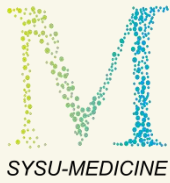


# Guidebook For Simple Synthetic Biology Experiment





**This guide book will offer a feasible approach for teachers in any educational services, especially high schools, to carry out several kinds of synthetic biology experiments so as to spark students' enthusiasm towards biology.**

**All the information shown below is designed on the basis of our successful activity named “secret of Synthetic biology”. Come with us, we will bring you into this amazing journey!**



**During this guide, we can complete three main experiments below:**

- **Extraction of DNA from buccal epithelial cells**
- **Polymerase Chain Reaction (PCR)**
- **Agarose Gel Electrophoresis**



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# Contents

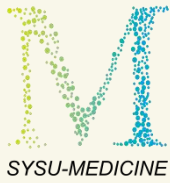
**1.What should you prepare?**

**2.How to launch each experiment?**

**3.Future**



&amp;



SYSU-MEDICINE

# 1.What should you prepare?

Experiment	Appliance	Amount	Example
Extraction of DNA from buccal epithelial cells	centrifuge	(1) speed $\geq$ 400rpm	1. Thermo
		(2) speed=12000rpm	2. Eppendorf Centrifuge 5430R
			3. Eppendorf Centrifuge 5810R
	thermostat (70℃)	1	CORNING
	micropipet	(1)100~1000 $\mu$ L	1. CORNING
		(2)10~200 $\mu$ L	2. Eppendorf
		(3)1~10 $\mu$ L	
	refrigerator (4℃)	1	
PCR	PCR System	1	1. S1000 Thermal Cycler 2.eppendorf 3.Mastercycler
Agarose Gel Electrophoresis	electrophoresis apparatus	1	POWER PAC BASIC
	microwave oven	1	
	Gel plates	1	
Others	lab-gown	1	

Experiment	Mertirial	Amount (per person)
Extraction of DNA from buccal epithelial cells	Normal Saline	30ml
	centrifuge tube (15ml)	1
	TIANGEN_DP304 DNA Kit	
	centrifuge tube (1.5ml)	3
PCR	PCR reaction solution	1
Agarose Gel Electrophoresis	Agarose powder	0.05g-0.10g
	DNA Dye	
	Marker	1 $\mu$ L
Others	lab-gown	1



&



## **2.How to launch each experiment?**

**——Here we design a teaching course for you,  
allowing you to easily approach the way to teach.**



# The secret of Synthetic biology



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**Overview**

**1**

**Experiments**

**2**

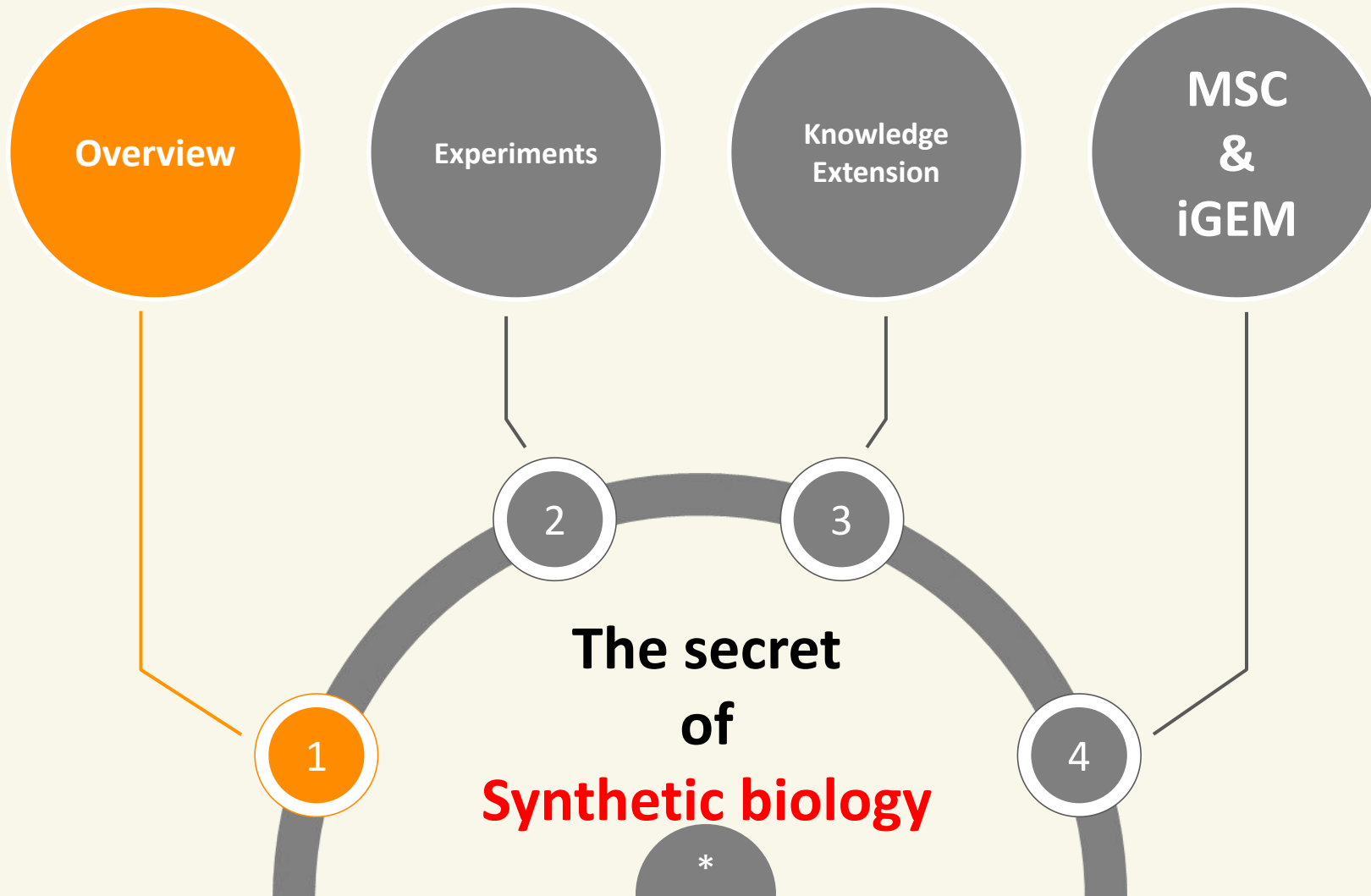
**Knowledge  
Extension**

**3**

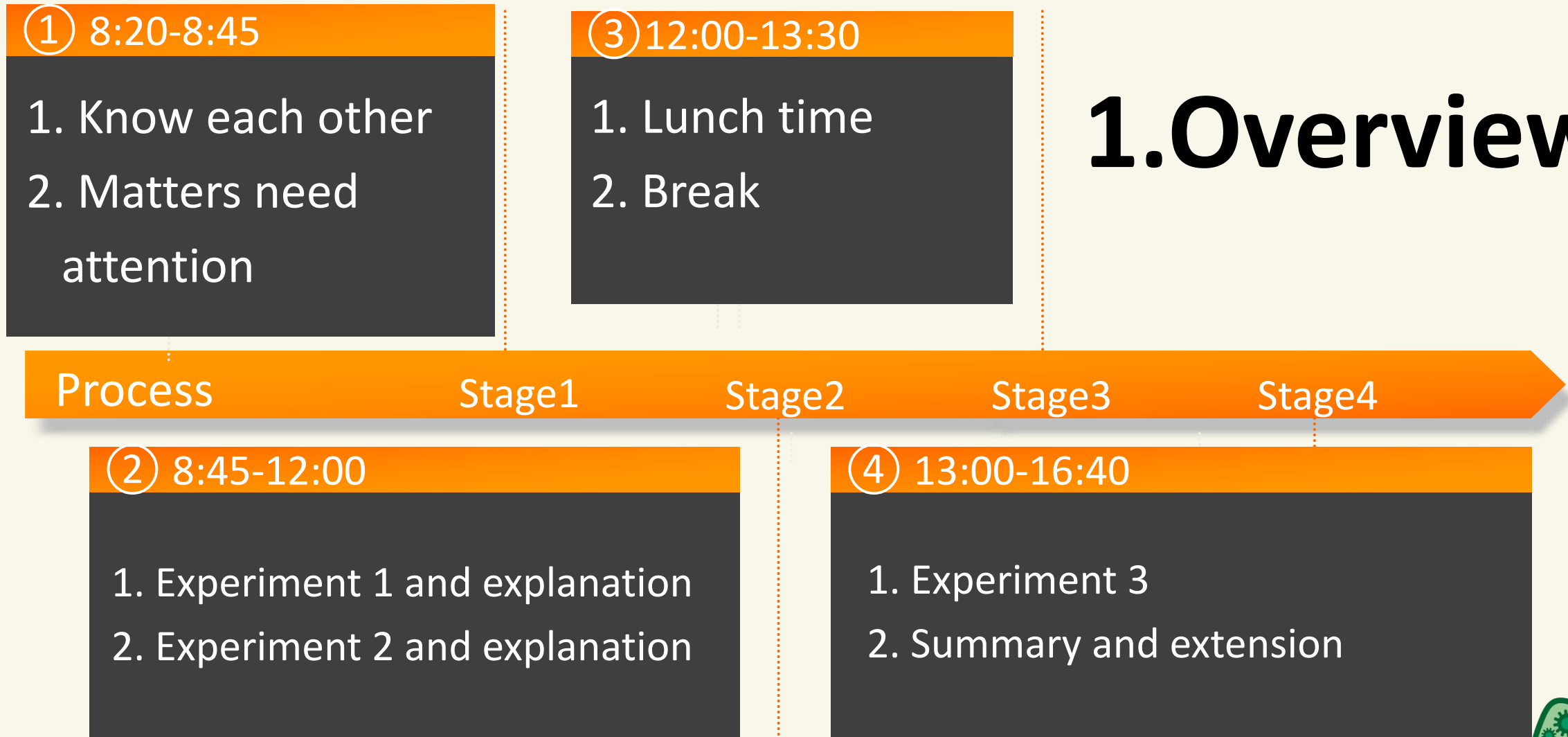
**MSC & iGEM**

**4**





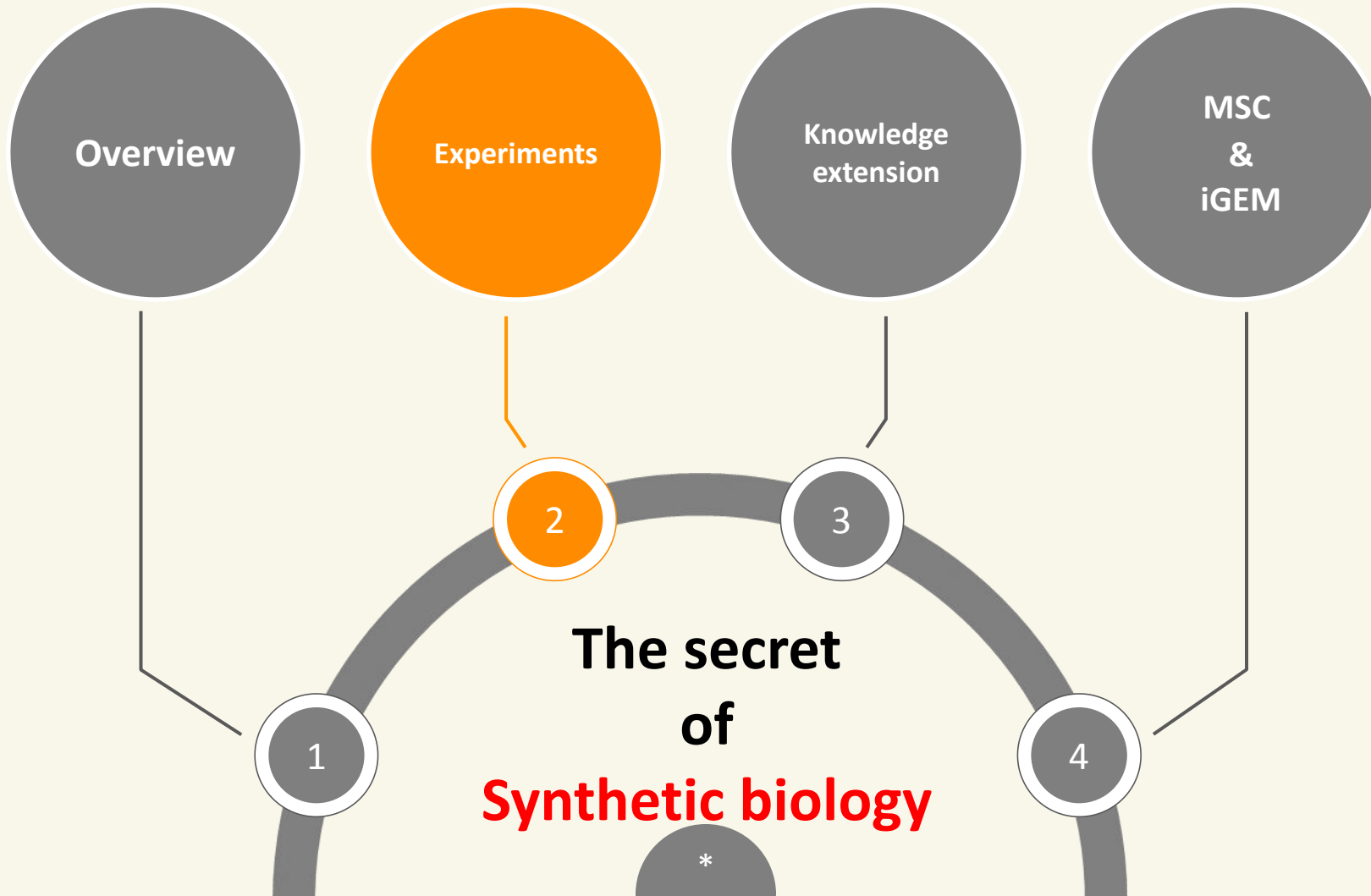
# 1. Overview



# Matters need attention

1. In the process of experiments, please keep quiet. Do not speak loudly.
2. Foods are banned in lab.
  - **Lab coats should be worn before entering lab.**
  - Instruments and materials should be counted. Do not hesitate to ask for replacement if there is any damaged instrument.
  - Please save reagents. Do not litter. Waste should be collected in designated box.
  - All the used instruments should be cleaned after experiments and the table top should be cleaned up.





## 2.Experiments

### Experiment 1

Extraction of DNA from  
buccal epithelial cells

# What are we extracting?

## Classification of nucleic acids

### □ DNA

mainly exists in the chromosome in cell nuclear. Some DNA exist outside of nuclear, including mitochondrial DNA (mtDNA), chloroplast DNA and plasmid DNA.

### □ RNA

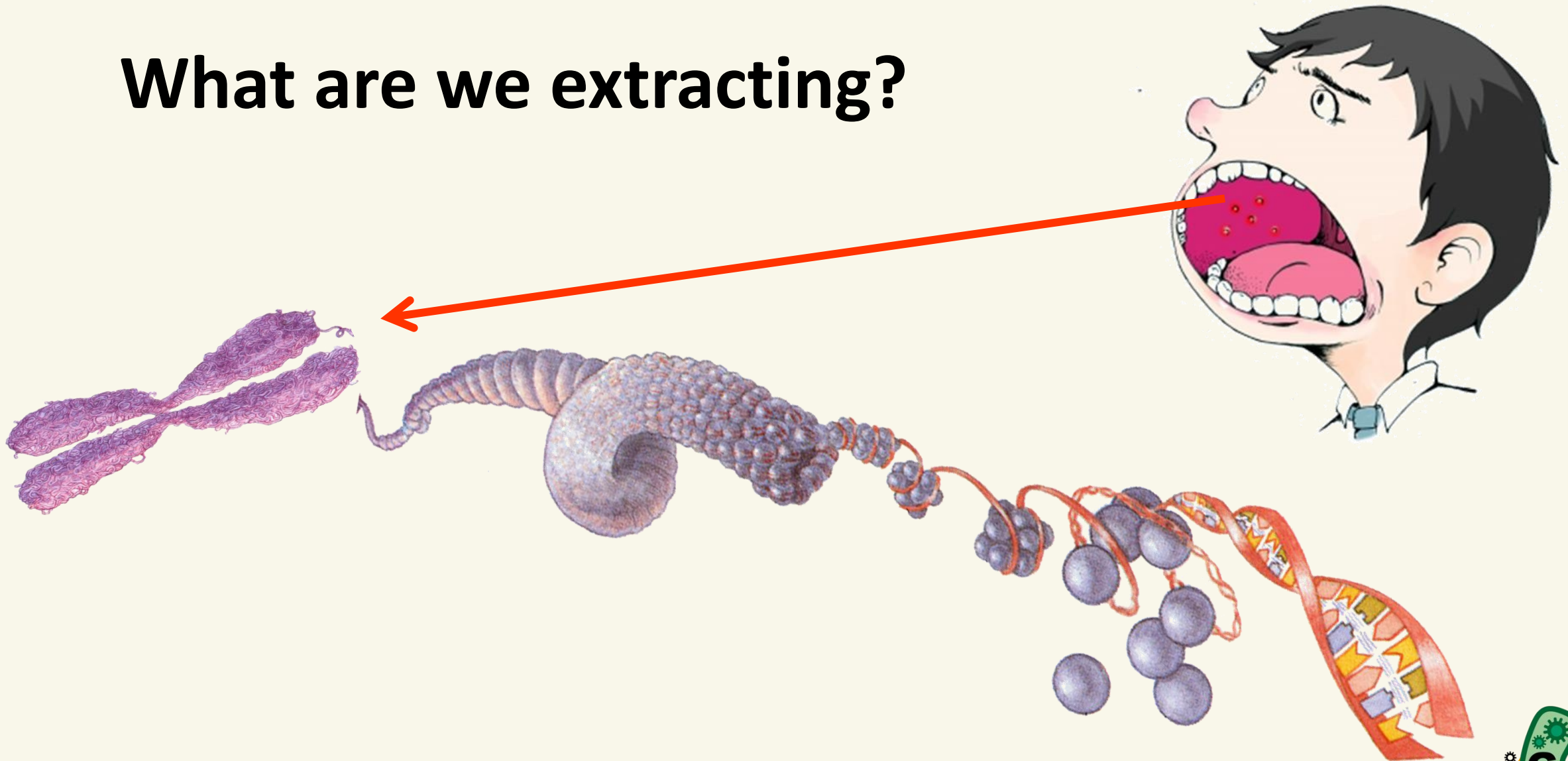
Exists in cytoplasm, like mRNA, rRNA, tRNA, etc.

- Apart from DNA and RNA mentioned above, non cellular virus and bacteriophages have only DNA or RNA.

**All nucleic acids in buccal epithelial cells are combined with proteins.**



# What are we extracting?



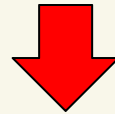
# Question:

How to extract DNA from buccal epithelial cells?



# Three core steps :

**1. Crush cells**



**2. Removal of impurities**



**3. Separate DNA out**

# 1. Crush cells

How can we crush cells?

# 1.Cell Disruption

**SDS (lauryl sodium sulfate )**

——Detergent for protein denaturation

## 2. Removal of impurities

### ( 1 ) What do impurities consist?

- ① Protein, Polysaccharide and lipid combined with proteins
- ② Other unwanted nuclear acid
- ③ Salt
- ④ organic solvent

### ( 2 ) How to get rid of proteins?

- ① Proteinase K hydrolyzes proteins
- ② Phenol and chloroform / isopentanol extract and separate proteins



# 3.Precipitate DNA

Ethanol causes DNA to precipitate from solution.



**However, we got  
more advanced  
method today!**



# Two important instruments

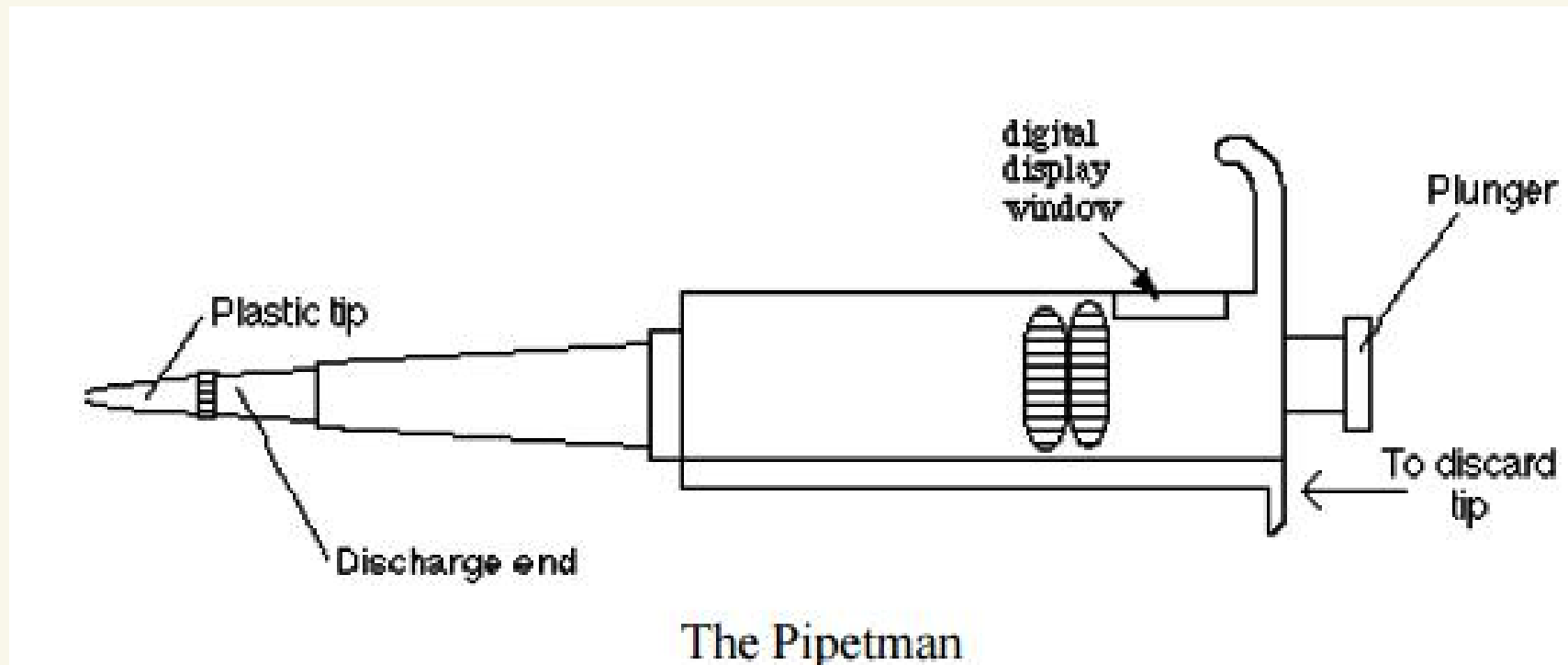
( 1 ) micropipet



( 2 ) Centrifuge



## Step 1: Overall observation





## Step 2: **Scale Value**

**Do not exceed the scale value when adjusting the pipette,  
otherwise it is easy to be damaged.**



20  $\mu$ l

100  $\mu$ l

1000  $\mu$ l



0.5--10 $\mu$ l20--200 $\mu$ l100--1000 $\mu$ l

1 0 . 0

2 0 0

1 0 0 0

## Step 3: Adjustment

Adjusting Knob



scale





## Step 4: **Use it**

**Attention:**

**The first gear:**

**Absorbtion**

**The second gear:**

**Blow out**



## Step 5: **Maintenance**

**1 Do not exceed the scale value when adjusting the pipette, otherwise it is easy to be damaged.**

**2 Do not disassemble the pipette**

**3 Pay attention to corrosive and toxic agents**

## ( 2 ) Precautions for the Use of Centrifuges

1. The samples should be pre-equilibrated when using a centrifugal.
2. Make sure all steps are correct, cover the inner cover, press the Start button and closely monitor the centrifugal.



# Nuclear DNA extraction adsorption column CB3

Similar principle, easier procedure.





# Experiments

1. Gargle with normal saline, mouthing 15ml of it for 3 minutes. Finally, collect the liquid and centrifuge at 5000 rpm for 4 minutes.



2. Collect a half of cell deposit to centrifuge tube, add **GA** buffer to 200  $\mu$ l.



3. Add 20  $\mu$ l Proteinase K, mix thoroughly by vortex



4. Add 200  $\mu$ l Buffer GB to the sample, mix thoroughly by vortex, and incubate at 70°C for 10 min to yield a homogeneous solution.



5. Add 200  $\mu$ l ethanol (96-100%) to the sample, and mix thoroughly by vortex for 15 s

# Experiments

6. Pipet the mixture from step 4 into the Spin Column CB3 and centrifuge at 12,000 rpm for 30 s.



7. Add 500  $\mu$ l Buffer GD (Ensure ethanol (96-100%) has been added) to Spin Column CB3, and centrifuge at 12,000 rpm for 1min, then discard the flow-through and place the spin column into the collection tube



8. Add 600  $\mu$ l Buffer PW (Ensure ethanol (96-100%) has been added) to Spin Column CB3, and centrifuge at 12,000 rpm for 1min. Discard the flow-through and place the spin column into the collection tube.

9. Repeat Step 8.



10. Place the Spin Column CB3 in a new clean 1.5 ml microcentrifuge tube



11. 5min and pipet 50-200  $\mu$ l Buffer TE directly to the center of the membrane. Incubate at room temperature (15-25°C) for 2-5 min



12. centrifuge for 2 min at 12,000 rpm.



13. Store it in refrigerator (4°C)

# Experiment 2

## Polymerase chain reaction (PCR)

- Background
- Definition
- Procedure
- Application
- Extension



# Background

- Motivation:  
Genetic disease  
Modify the gene  
How to obtain DNA  
How to amplify



Down's syndrome

## Background

In 1953, James Watson and Francis Crick —double helix structure of DNA

In 1958,—mechanism of DNA amplification  
Matthew Stanley Meselson & Franklin on



Kary Banks Mullis

- Amplification of DNA
- High temperature--- ( replacement ) ---Helicase
- High temperature--- ( inactivation ) ---Polymerase
- DNA Polymerase produced by Thermophilic bacteria
- Primer





# Background

## Nobel Prize in Chemistry



Kary Banks Mullis



# Definition

## Polymerase Chain Reaction

a technique used in molecular biology to amplify a single copy or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

Cheaper and Convenient  
for Gene Identification  
in vitro



# 3 steps and 30 cycles for PCR

STEP 1.

- Denaturation

STEP 2.

- Anneal

STEP 3.

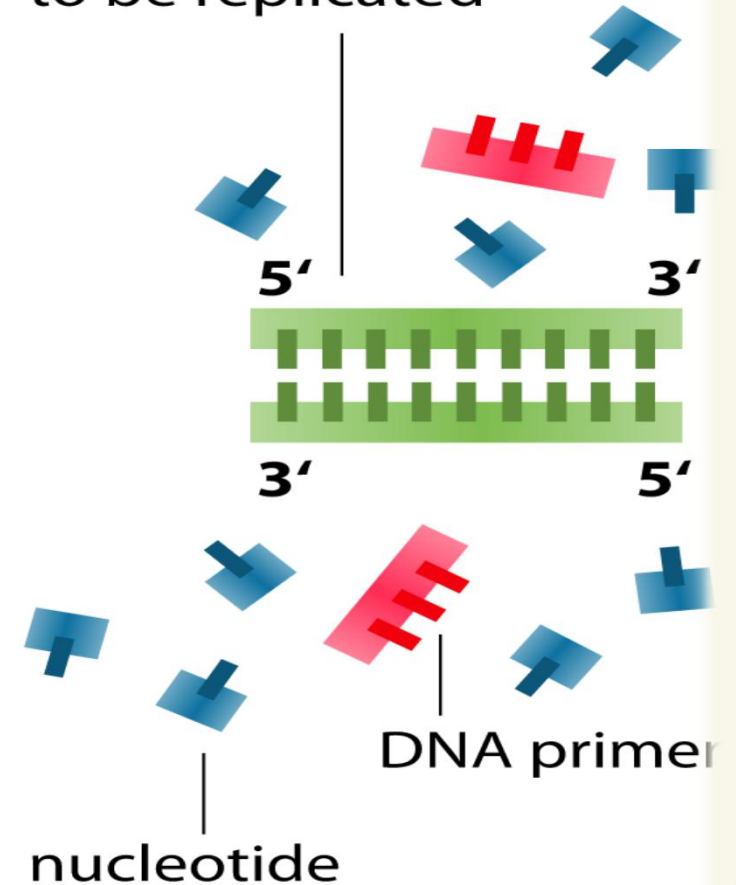
- Extend

30 cycles, billions of copies

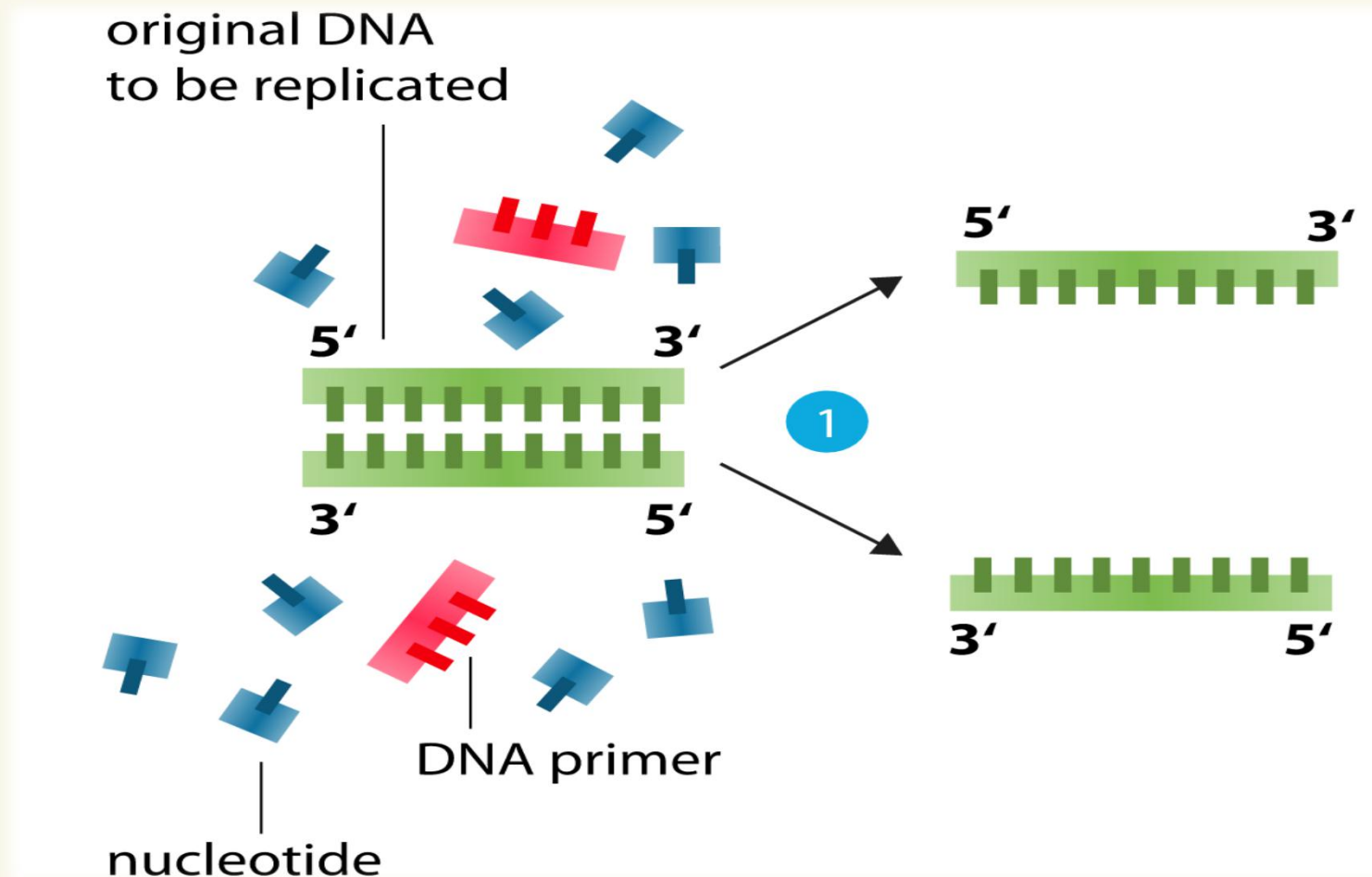
# Materials

- DNA template
- primer
- DNA polymerase
- dNTP
- Buffer
- ddH<sub>2</sub>O

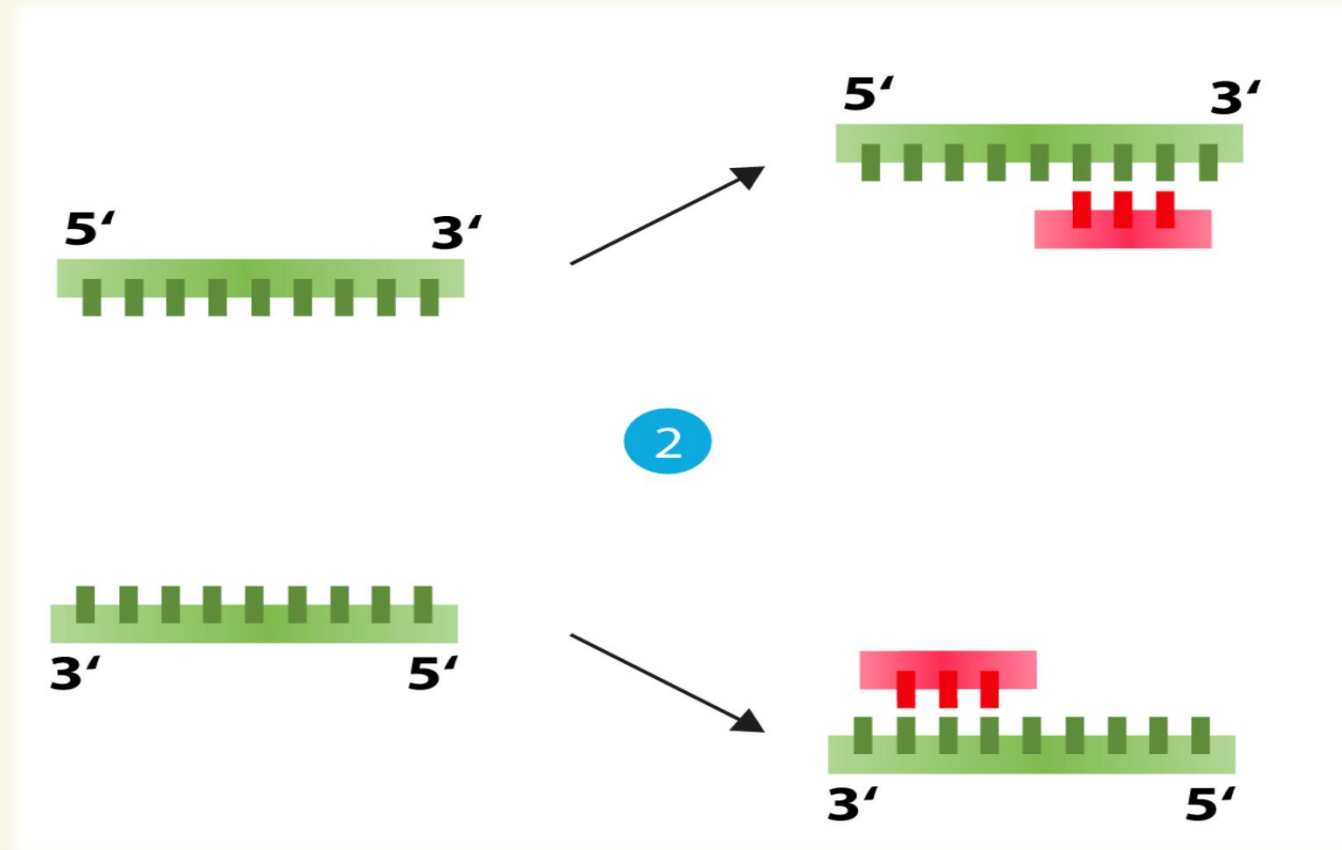
original DNA  
to be replicated



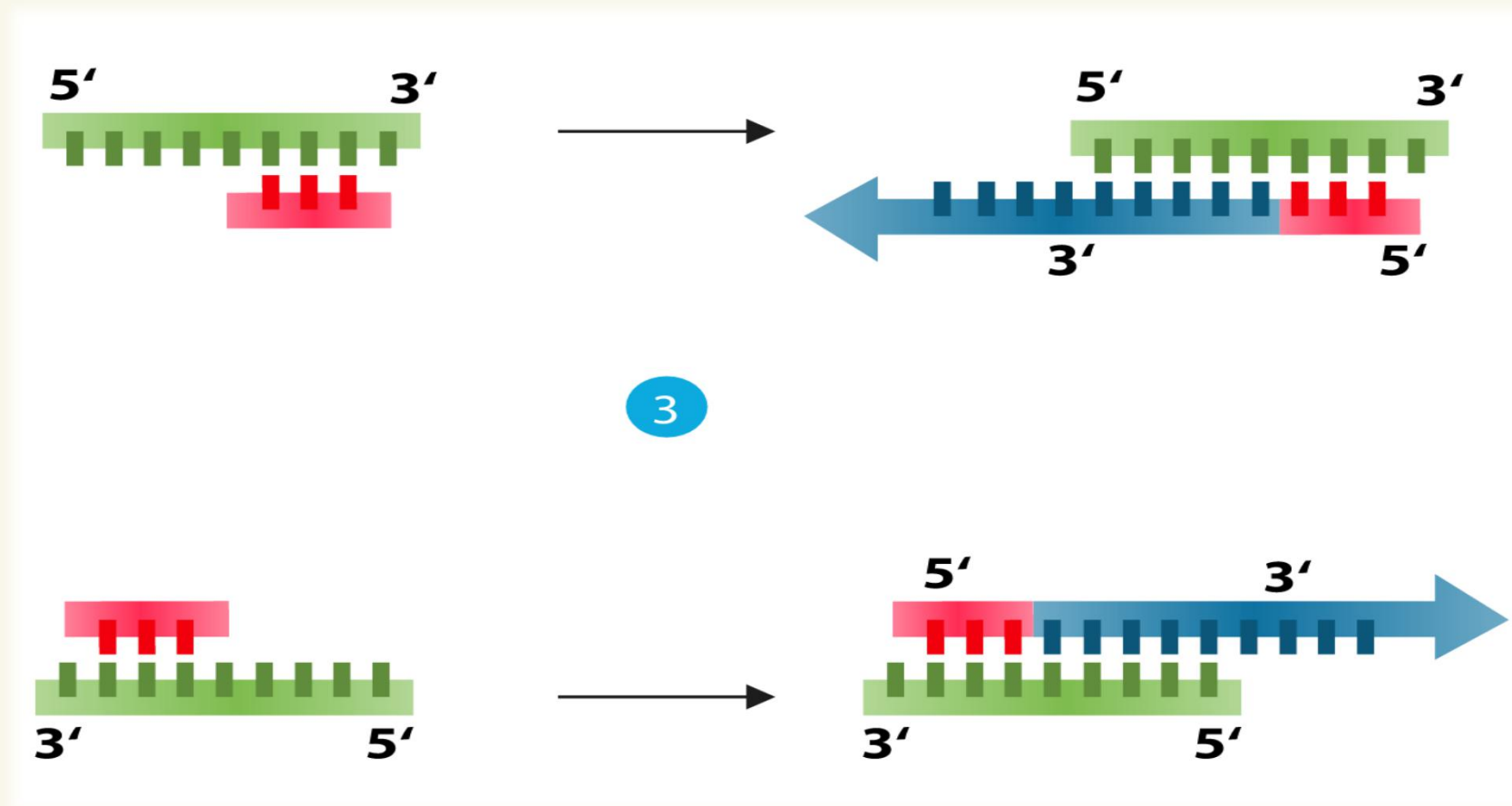
# Step 1. Denaturation



# Step 2. Anneal

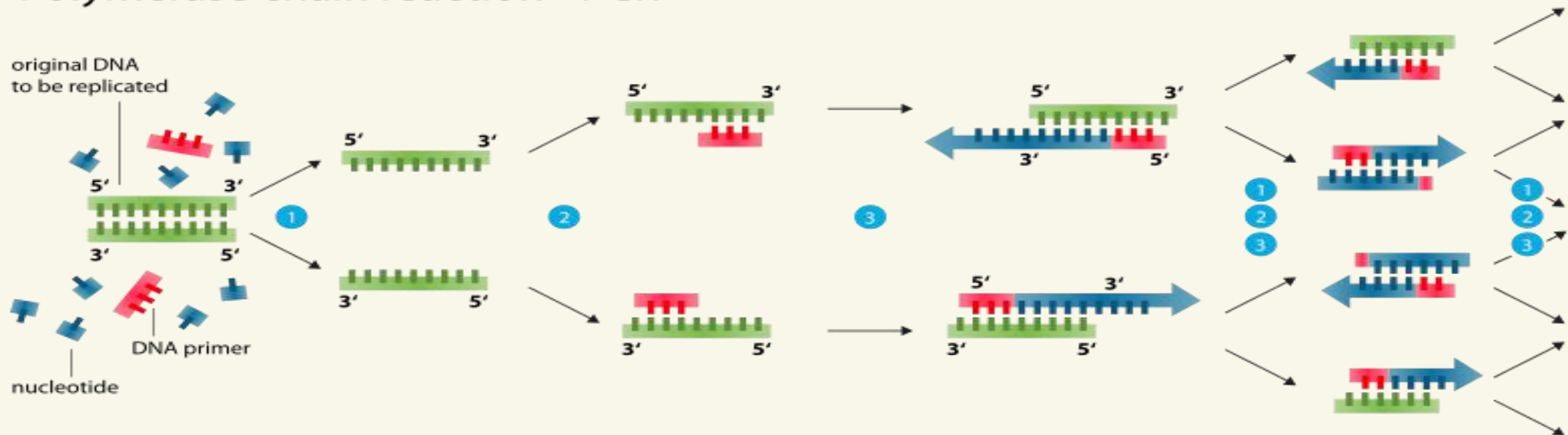


# Step 3. Extend



# Procedure

## Polymerase chain reaction - PCR

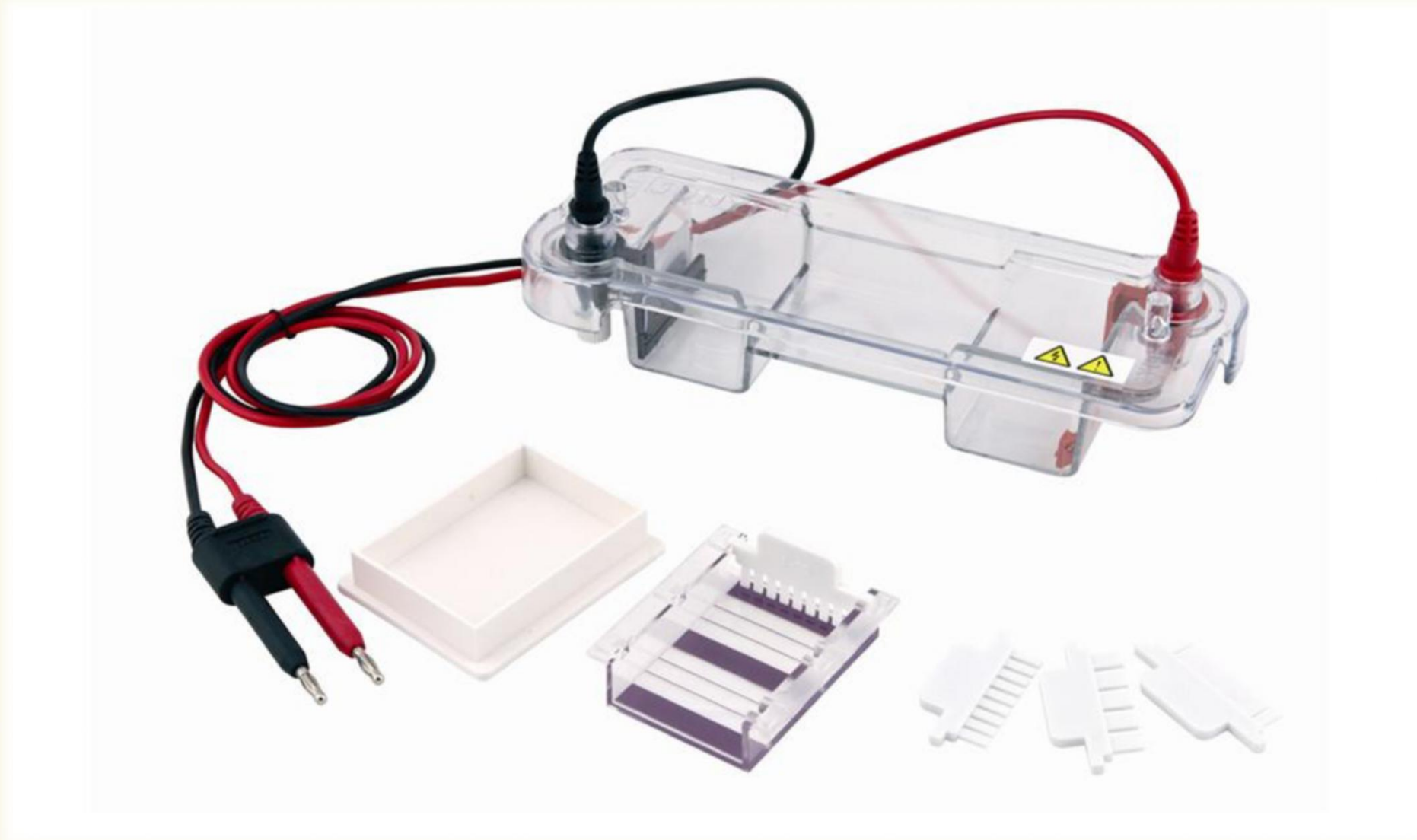


- 1 **Denaturation** at 94-96°C
- 2 **Annealing** at ~68°C
- 3 **Elongation** at ca. 72 °C

# Experiment 3

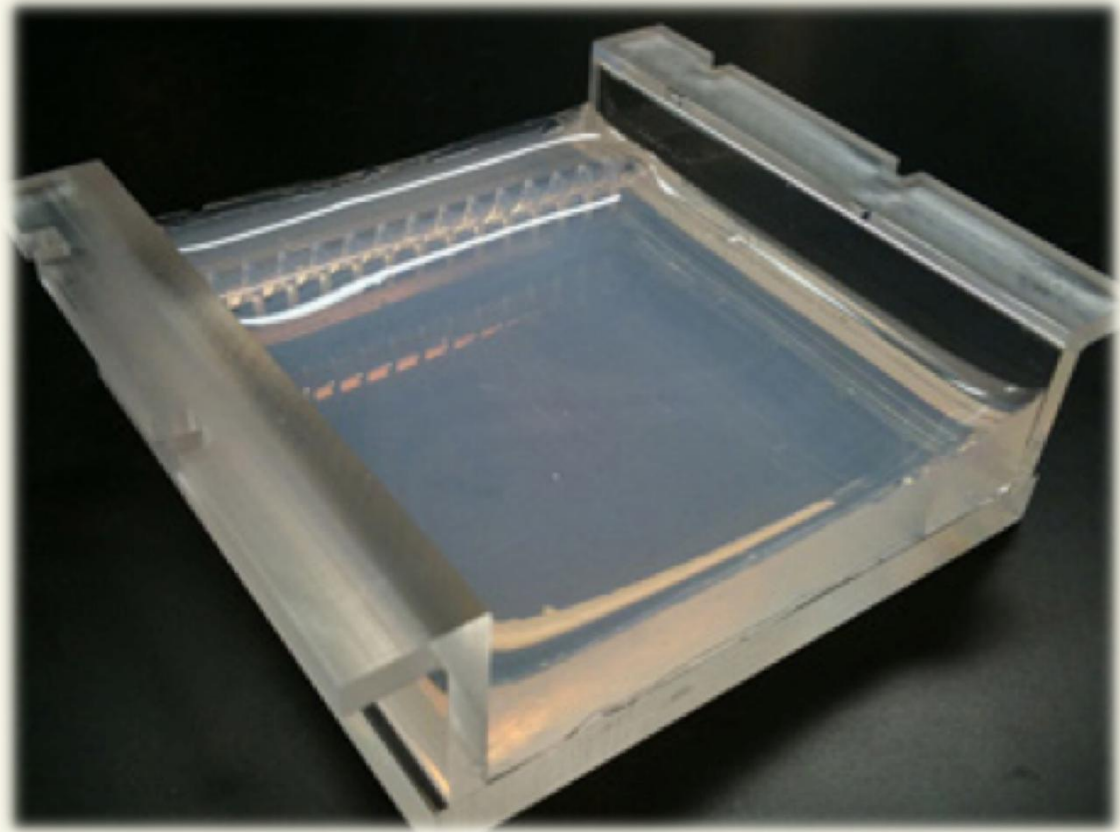
## Agarose Gel Electrophoresis





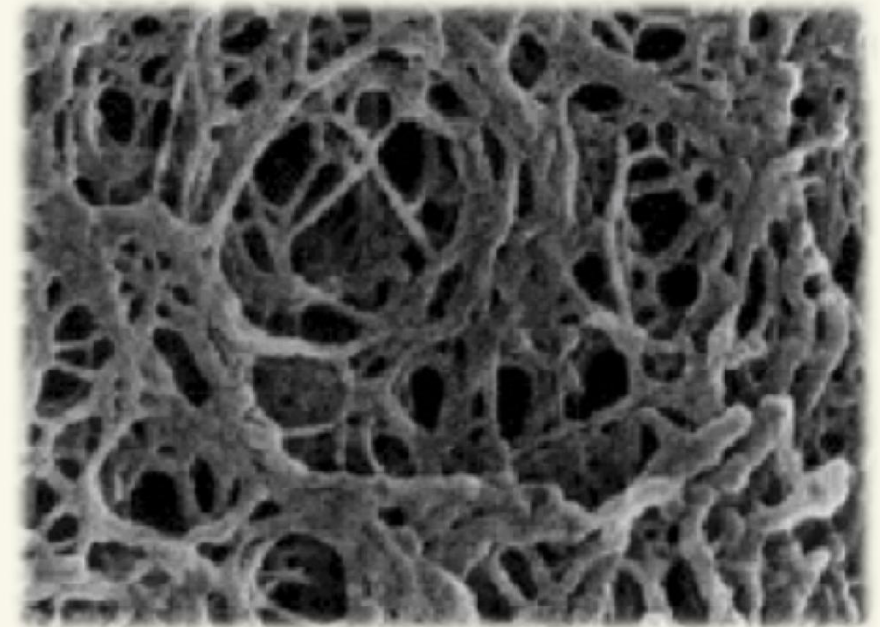
## Two effects

- Molecular sieve effect
- Charge effect



# Molecular sieve effect

Since agarose gel has a network structure, molecules would be resisted when going through the gel.



# Molecular sieve effect

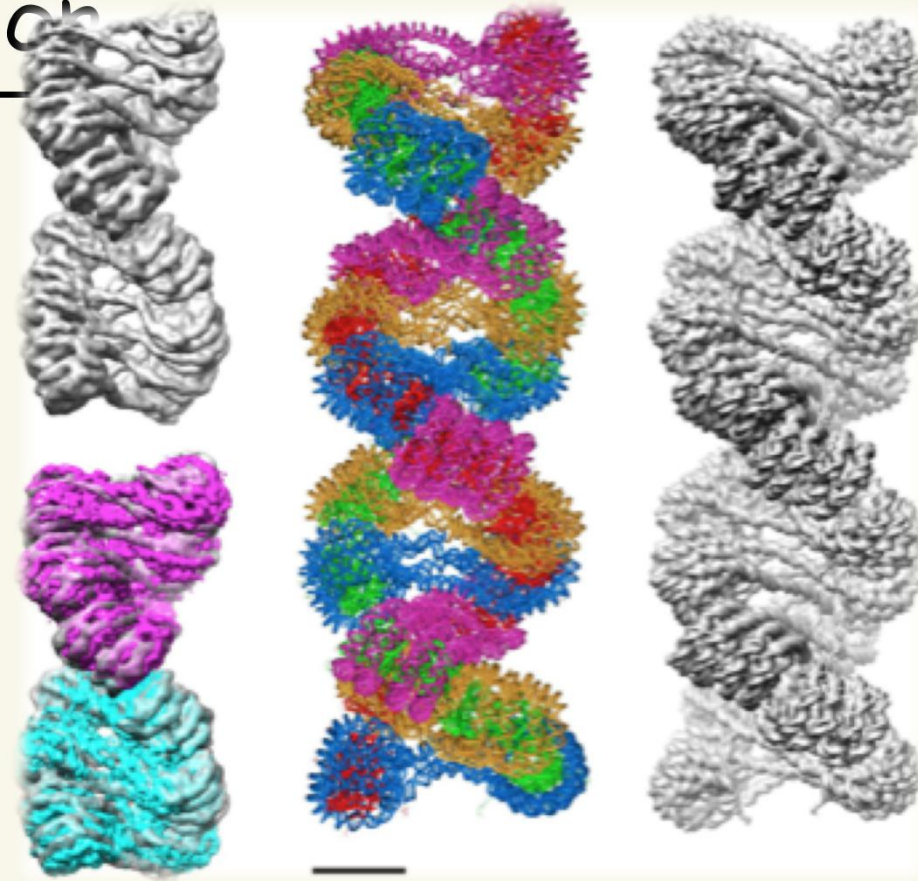
- Size of Molecule
- Size of dsDNA ↑ →  
Resistance ↑ →  
Slower



# Molecular sieve effect

- Molecular conformation

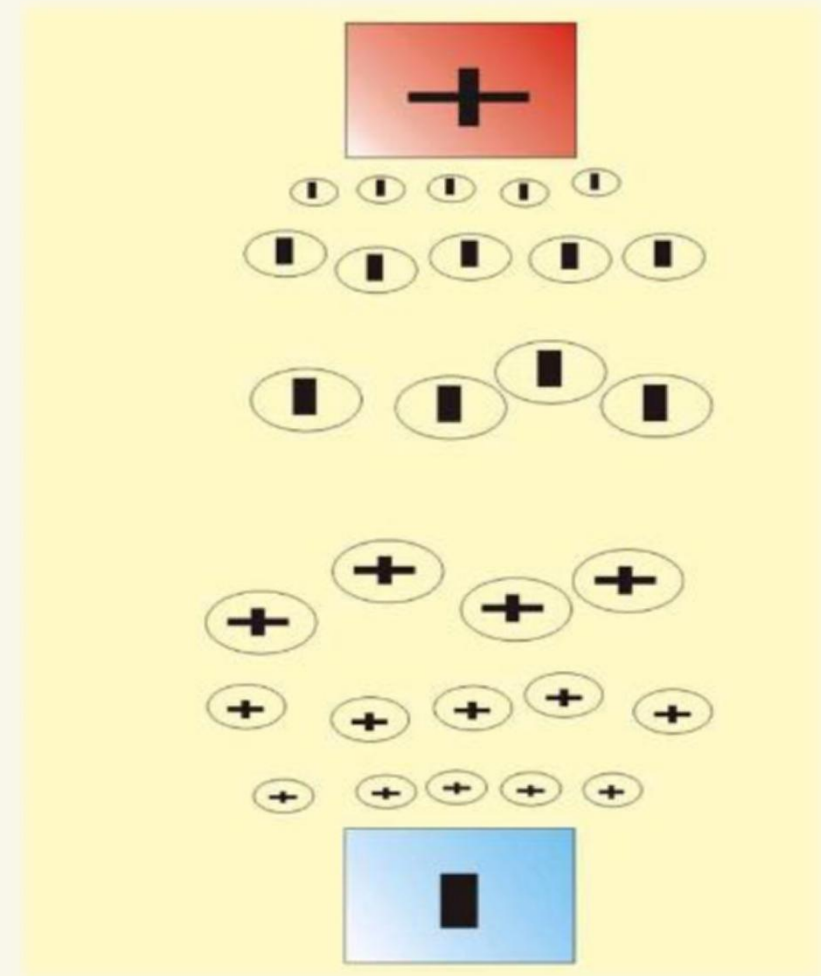
Supercoiling Structure > Linear Structure





# Charge effect

- Electrophoresis is the motion of dispersed particles relative to a fluid under the influence of a spatially uniform electric field. The technique applies a negative charge so proteins move towards a positive charge. This is used for both DNA and RNA analysis.



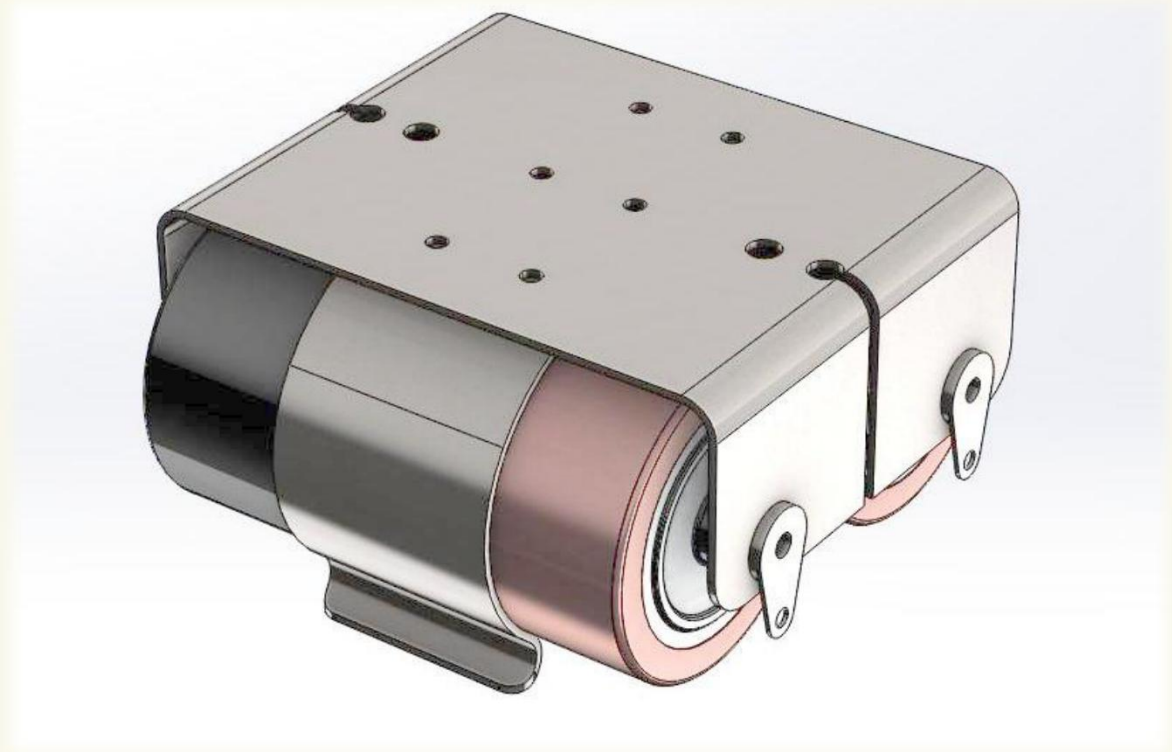
# Charge effect

- Supply Voltage;

Supply Voltage ↑

Speed ↑

Resolution ↓



# Charge effect

- Ionic strength

ddH<sub>2</sub>O ?

10\*Buffer ?

Buffer ✓





# Procedure

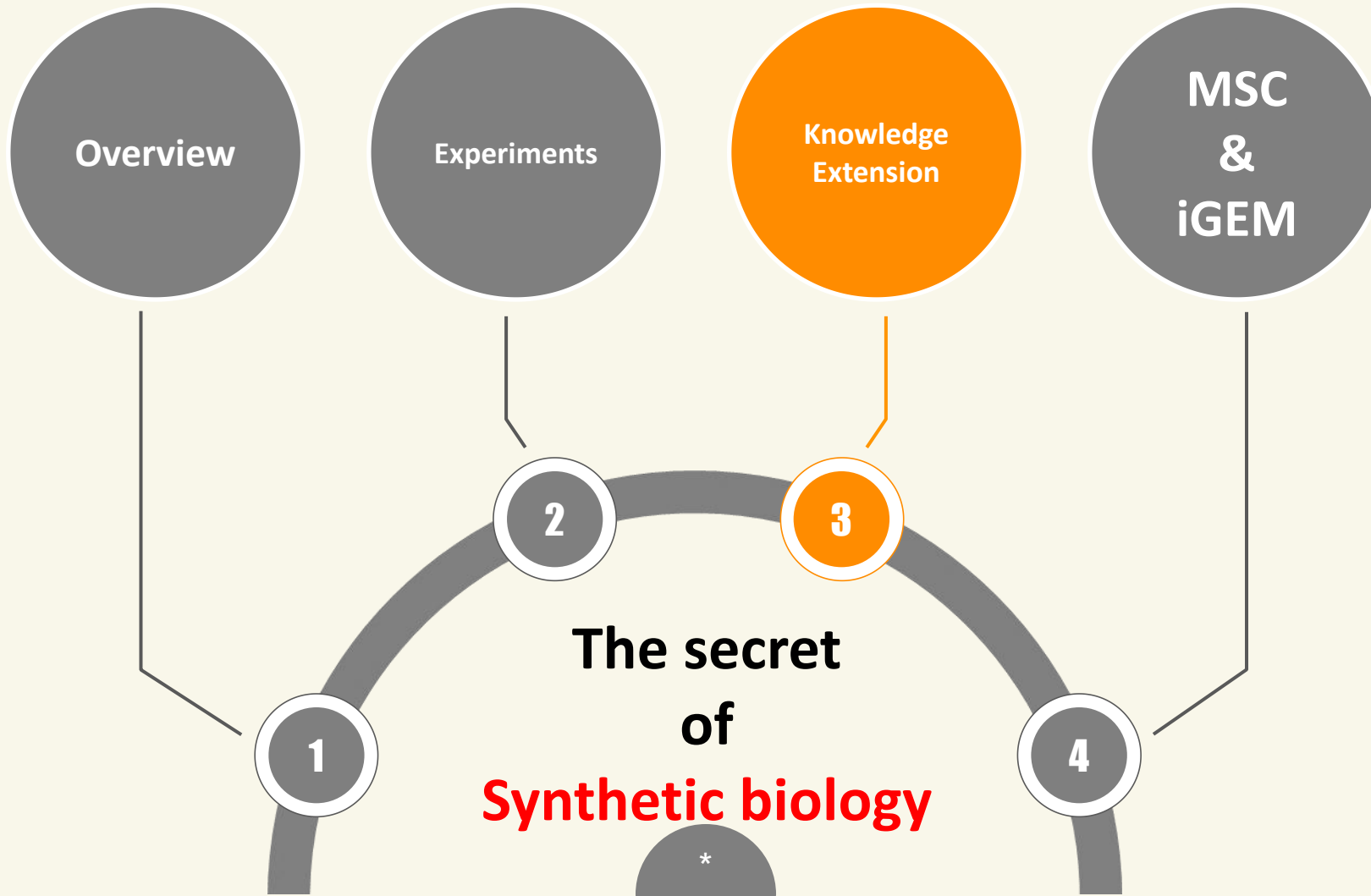
Preparation of  
gel



Sampling of  
DNA



Electrophoresis

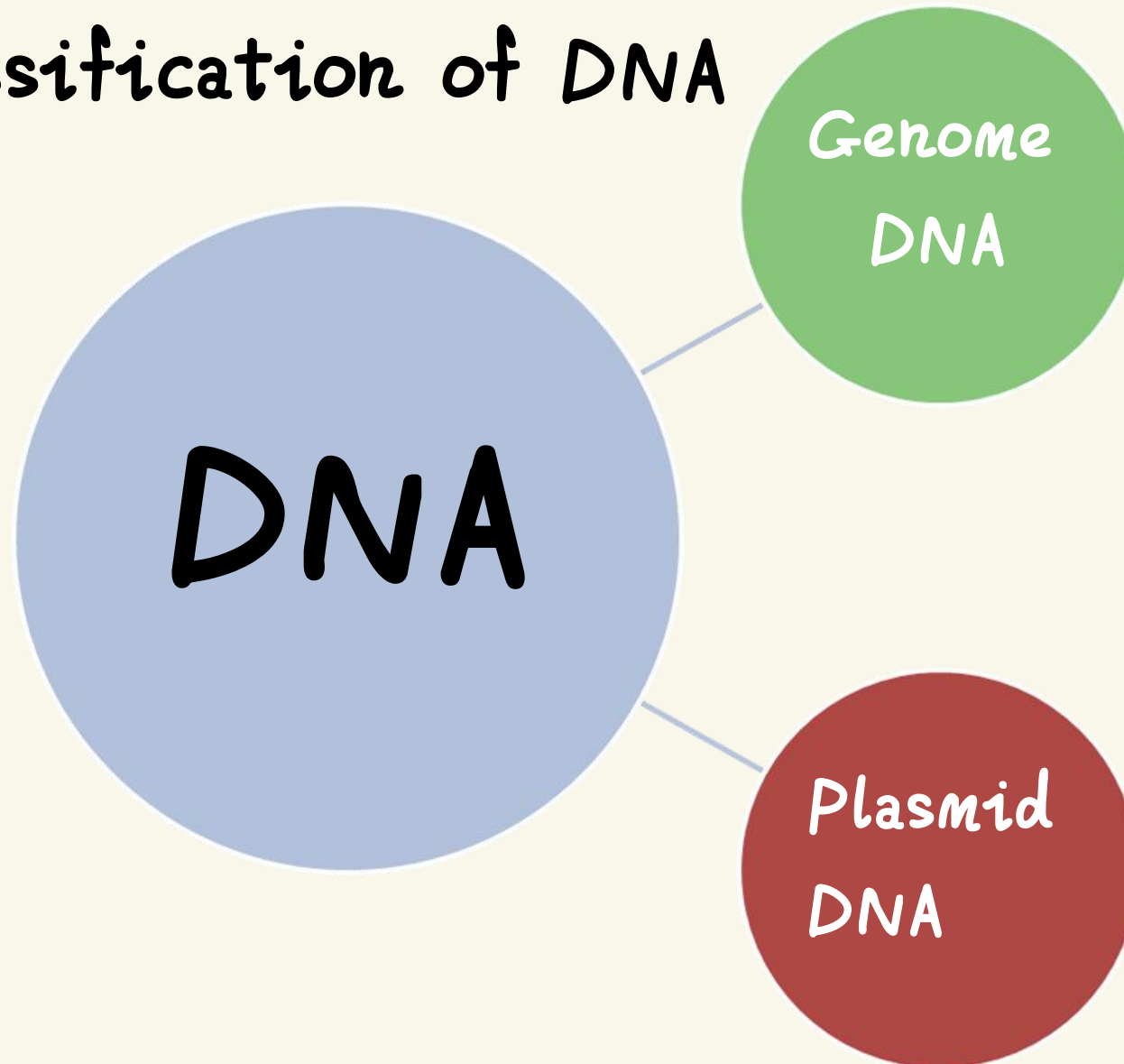


**01***Classification  
of DNA***02***Classification  
of PCR***03***Application  
of PCR*

## 3. Extension

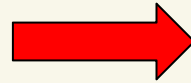
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# Classification of DNA



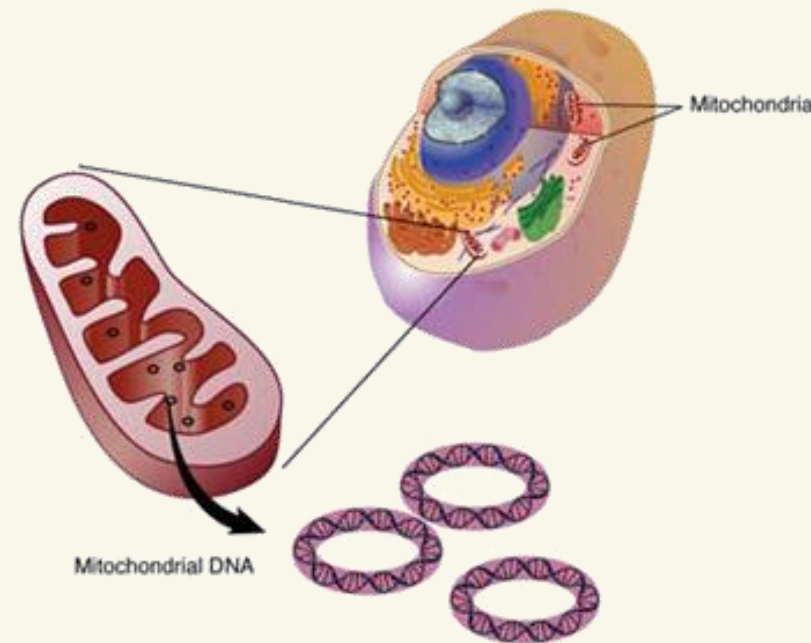
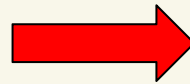
# Genome DNA

Nuclear genome



DNA of Human oral  
epithelial cells

Mitochondrial genome



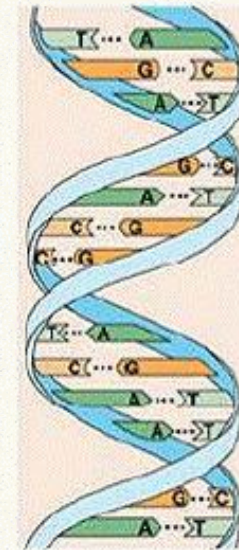
# Genome DNA

*How to extract  
mitochondrial genome?*

# Plasmid DNA

## Application:

- the first step of gene cloning (isolated from the carrier cells containing the gene of interest);
- Prepare for gene recombination;
- transfect into cells;
- expression of gene of interest

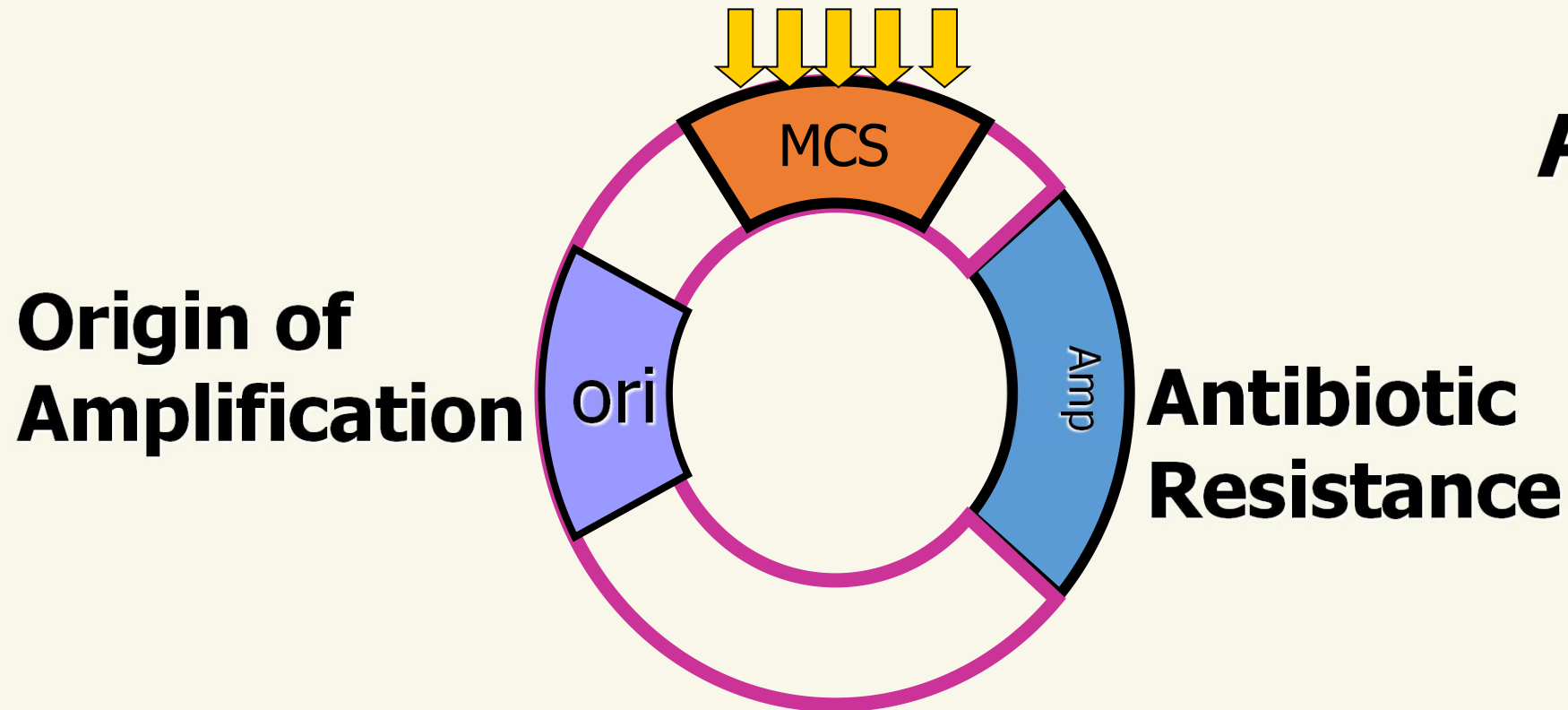


染色体DNA

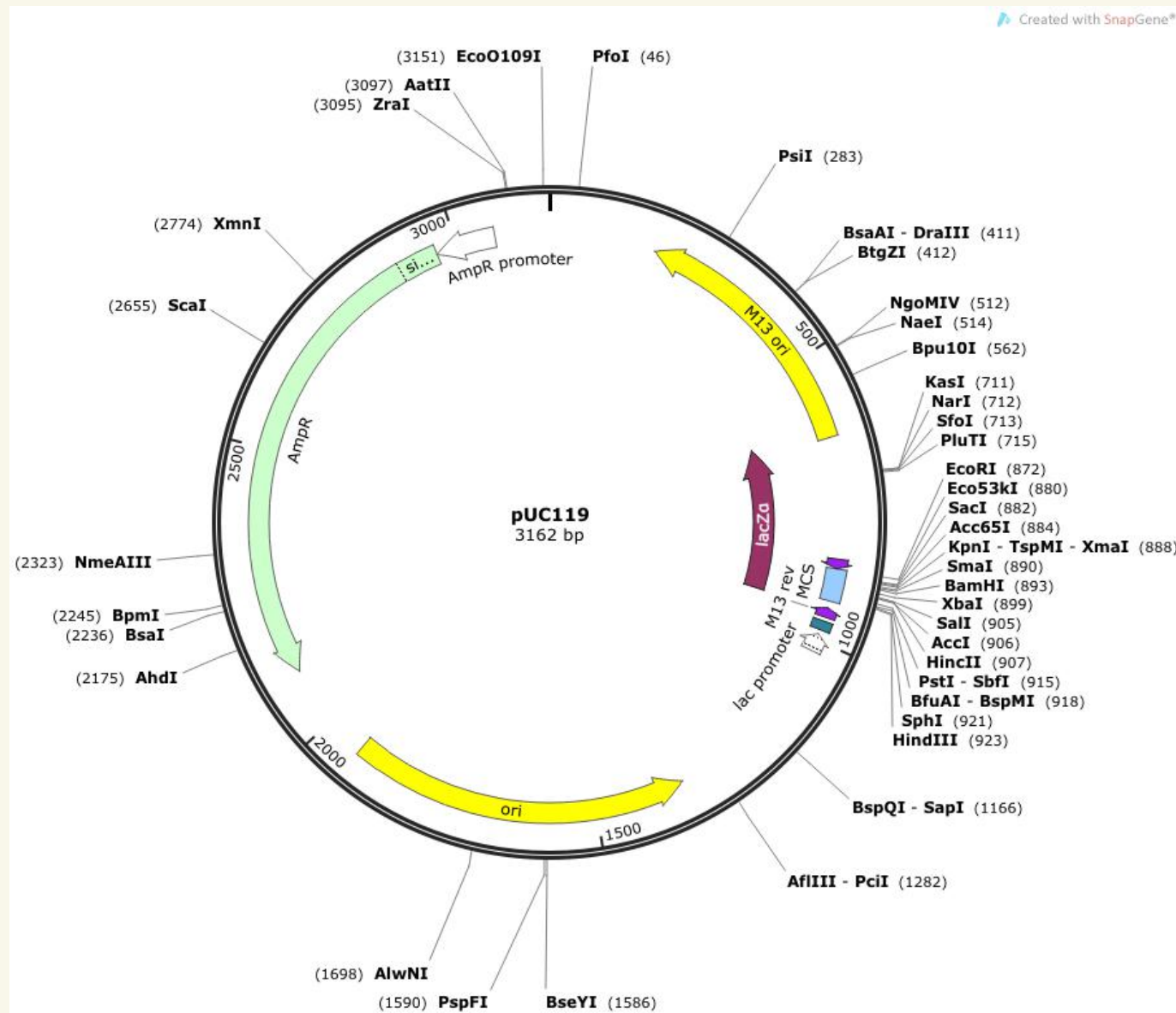


质粒DNA

**MCS (multiple cloning site)**







# Plasmid DNA

# Classification of PCR

1

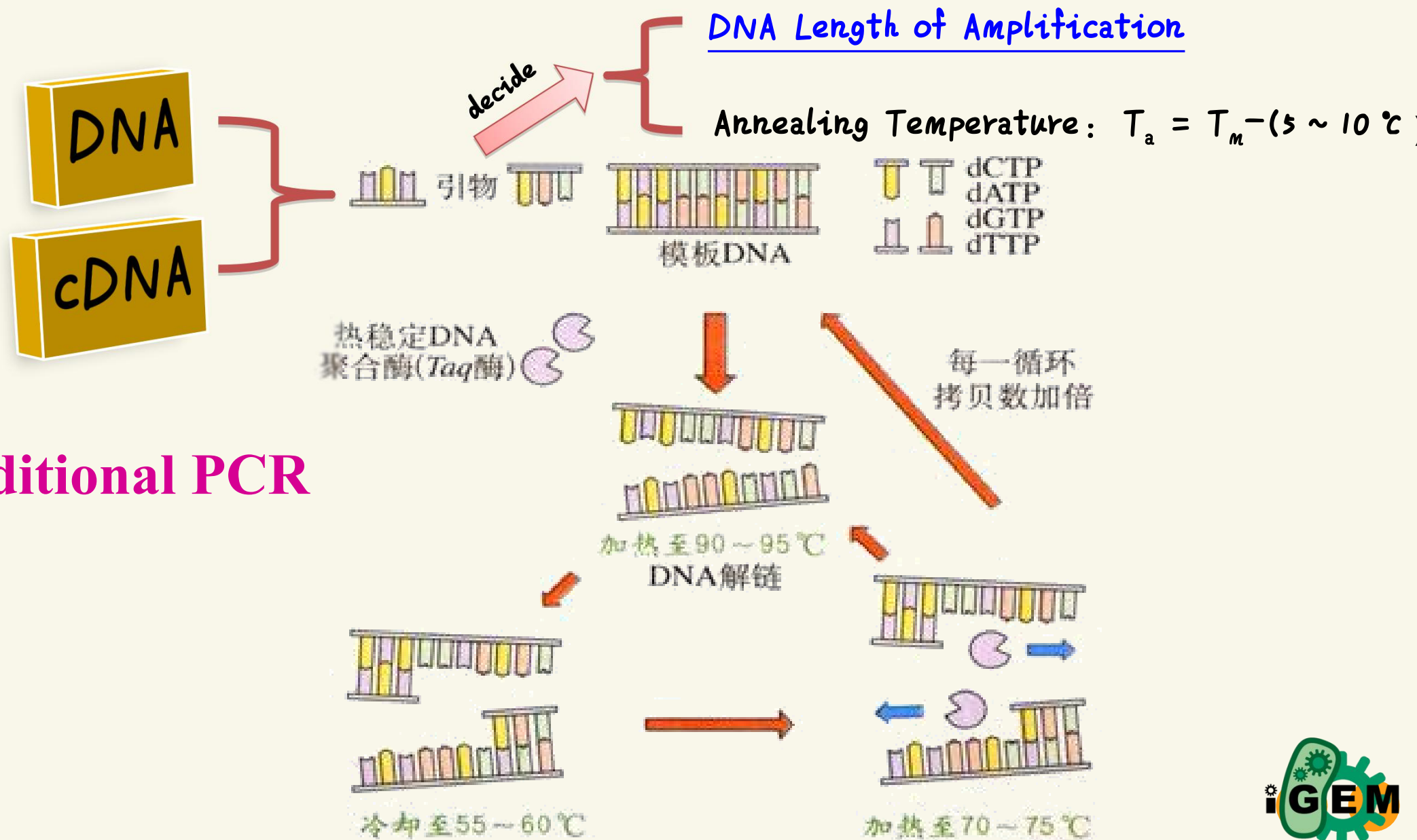
Traditional PCR

2

RT-PCR

3

Real time-PCR



## Attention:

94°C预变性	5分钟
94°C变性	30秒
57°C退火	30秒, 共30个循环
72°C延伸	30秒
72°C延伸	5分钟

*Primer*

*DNA Product*

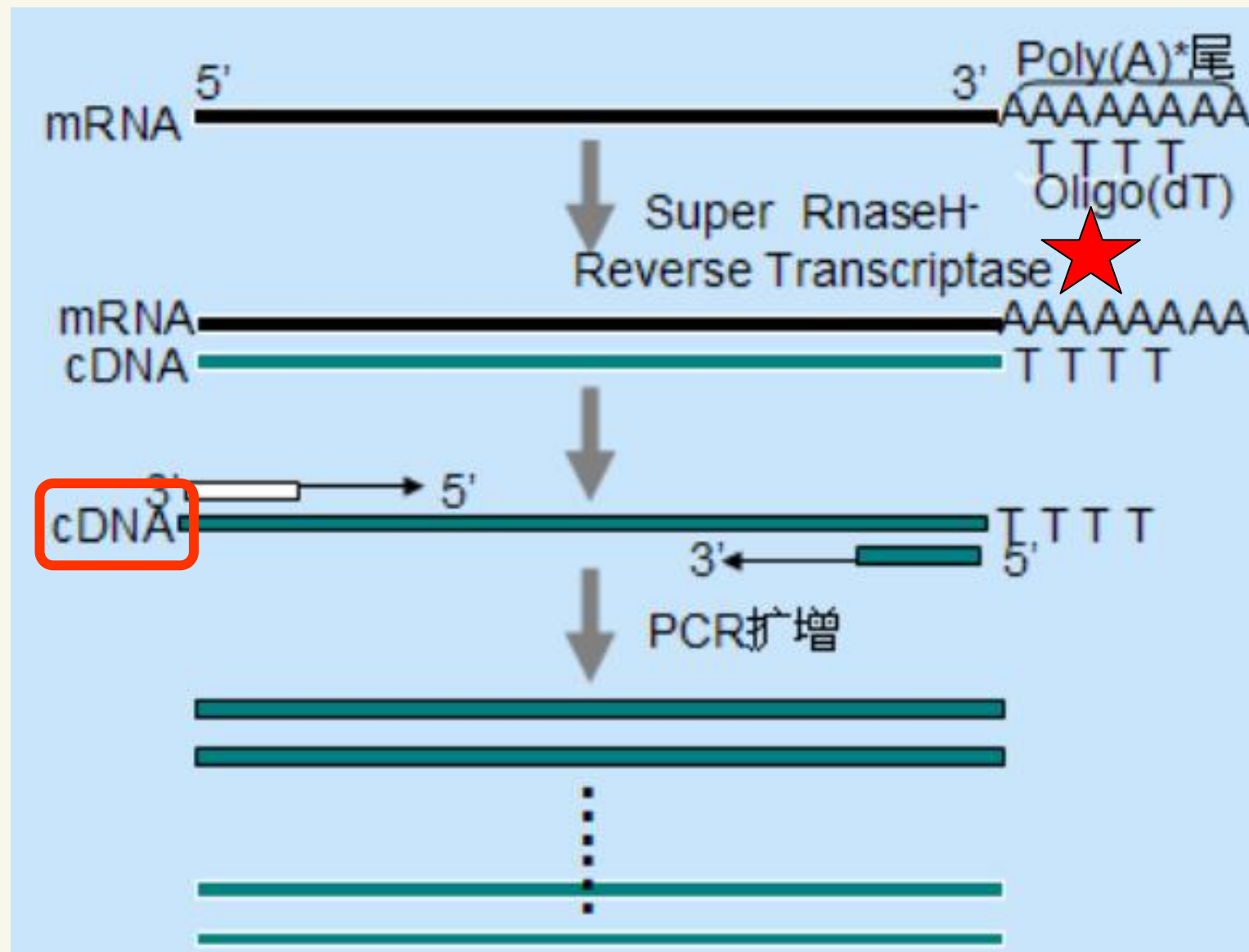
*decide*

*decide*

## (2) RT-PCR

- **Reverse transcription PCR (RT-PCR) is used to clone expressed genes by reverse transcribing the RNA of interest into its DNA complement through the use of reverse transcriptase. Subsequently, the newly synthesized cDNA is amplified using traditional PCR.**
- **RT-PCR is used to qualitatively detect gene expression through creation of complementary DNA (cDNA) transcripts from RNA.**

# RT-PCR



### (3) Real time-PCR

- **Traditional PCR is considered as semi-quantitative technology and could not accurately determine the initial concentration of the DNA template;**
- **qPCR is used to quantitatively measure the amplification of DNA using fluorescent dyes. qPCR is also referred to as quantitative PCR, quantitative real-time PCR, and real-time quantitative PCR.**
- **Two common methods for the detection of PCR products in real-time PCR are:**
  - **non-specific fluorescent dyes that intercalate with any double-stranded DNA**
- (2) sequence-specific DNA probes consisting of oligonucleotides that are labelled with a fluorescent reporter which permits detection only after hybridization of the probe with its complementary sequence.**





### (3) Real time-PCR & Traditional PCR

- Real-time monitoring

- The whole process of sample amplification and the increase of the product could be monitored in real time. In addition, the logarithmic phase of the reaction is visually observed

- Reduce the non-specificity of the reaction

- The specificity of the PCR reaction was enhanced by using primer and fluorescent probe to bind to the template at the same time

- Increase accuracy of quantity

- Quantitative by accurate algorithm.

- The result analysis is quicker and more convenient without agarose gel electrophoresis

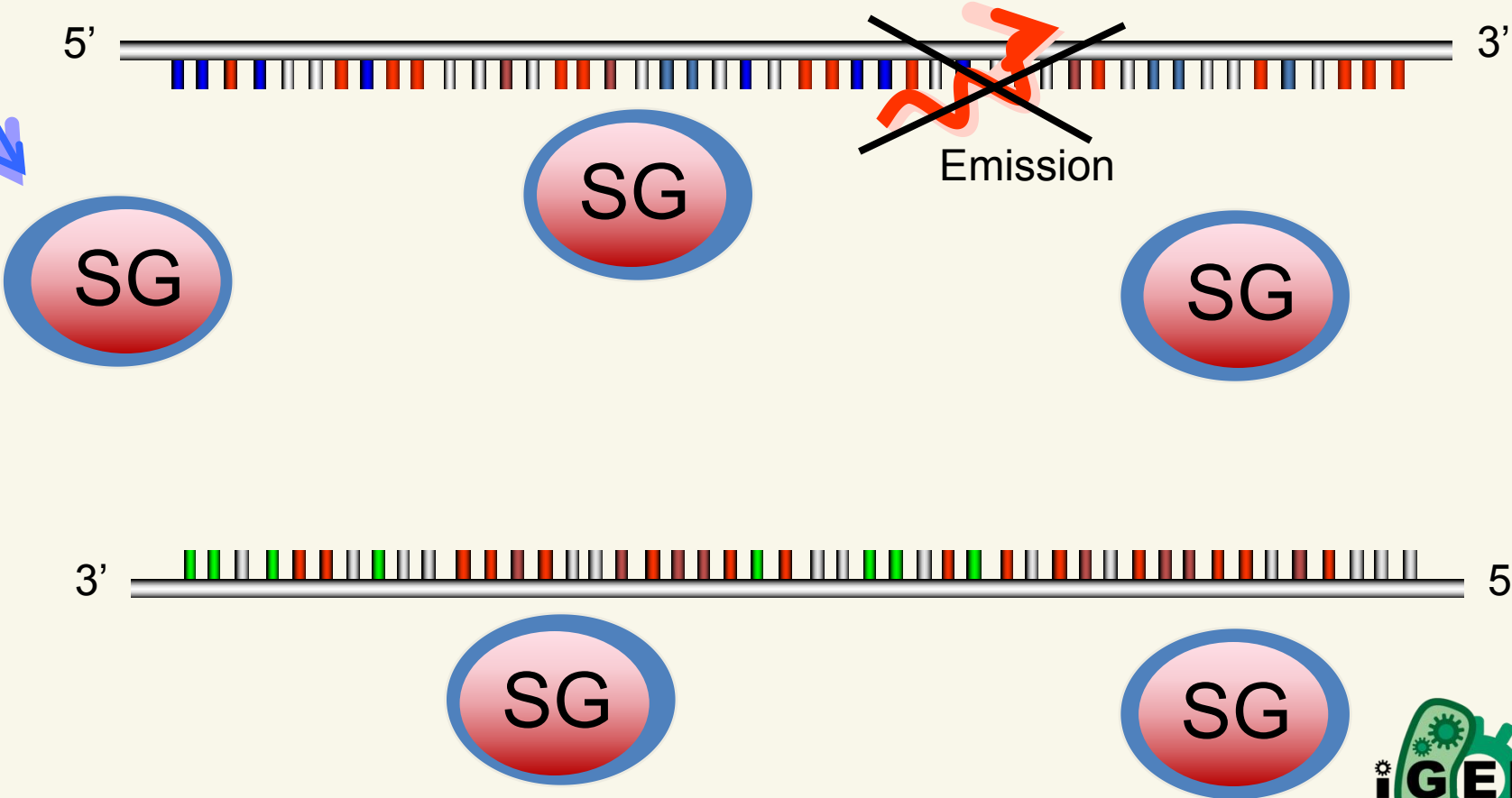


### (3) Real time-PCR - Non-probe real-time PCR

- A fluorescent dye capable of binding to double-stranded DNA is added;
- SYBR Green could bind to region of dsDNA double helix and fluorescence intensity could increase by 1000 times
- **Advantages:** Higher sensitivity, easier operating and lower cost;
- **Disadvantages:** The fluorescent dye could bind to all dsDNA, which slightly lower specificity and accuracy.

# SYBR-Green I

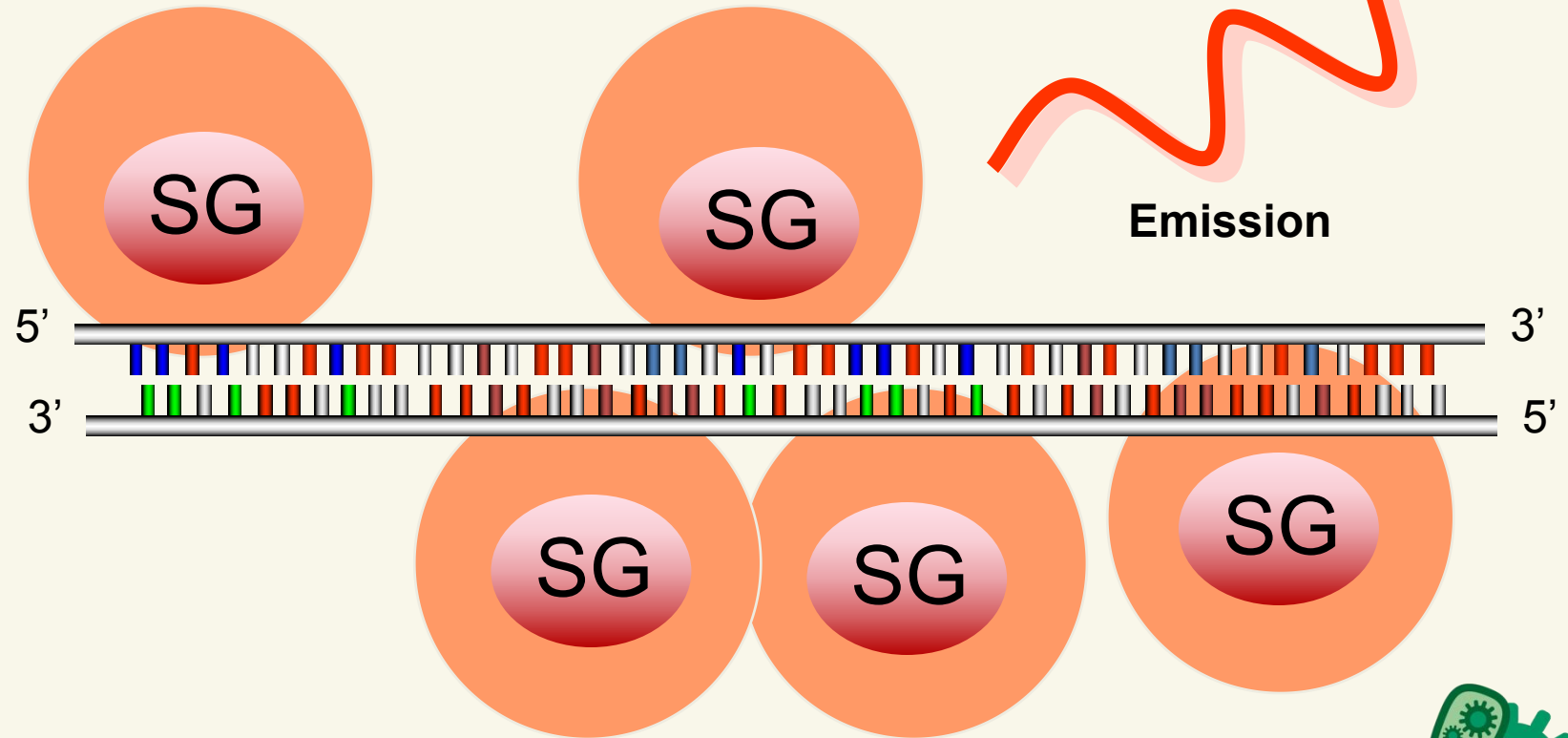
Excitation



# SYBR-Green I

Excitation

Emission



### (3) Real time-PCR - Probe real-time PCR

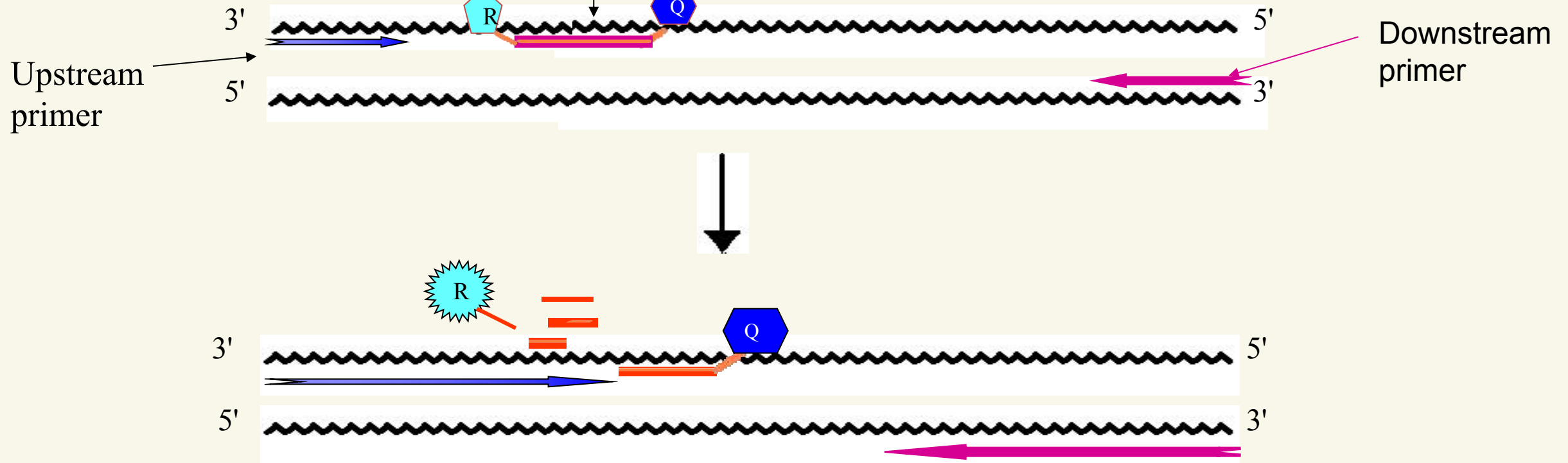
- The probe could be used to monitor PCR products in real time and bind to the region of template DNA to be amplified
- Significantly enhance specificity and accuracy



# Taqman Probe

- The probe could specifically bind to the region of template DNA to be amplified.
- A DNA-based probe with a fluorescent reporter at one end and a quencher of fluorescence at the opposite end of the probe.
- The close proximity of the reporter to the quencher prevents detection of its fluorescence; breakdown of the probe by the 5' to 3' exonuclease activity of the Taq polymerase breaks the reporter-quencher proximity and thus allows unquenched emission of fluorescence, which can be detected after excitation with a laser.
- An increase in the product targeted by the reporter probe at each PCR cycle therefore causes a proportional increase in fluorescence due to the breakdown of the probe and release of the reporter.

fluorescently-labeled primer



# Application of PCR

1

PCR Product

2

Analysis

# (1) Overexpression-iPS Technology

PCR  
Product

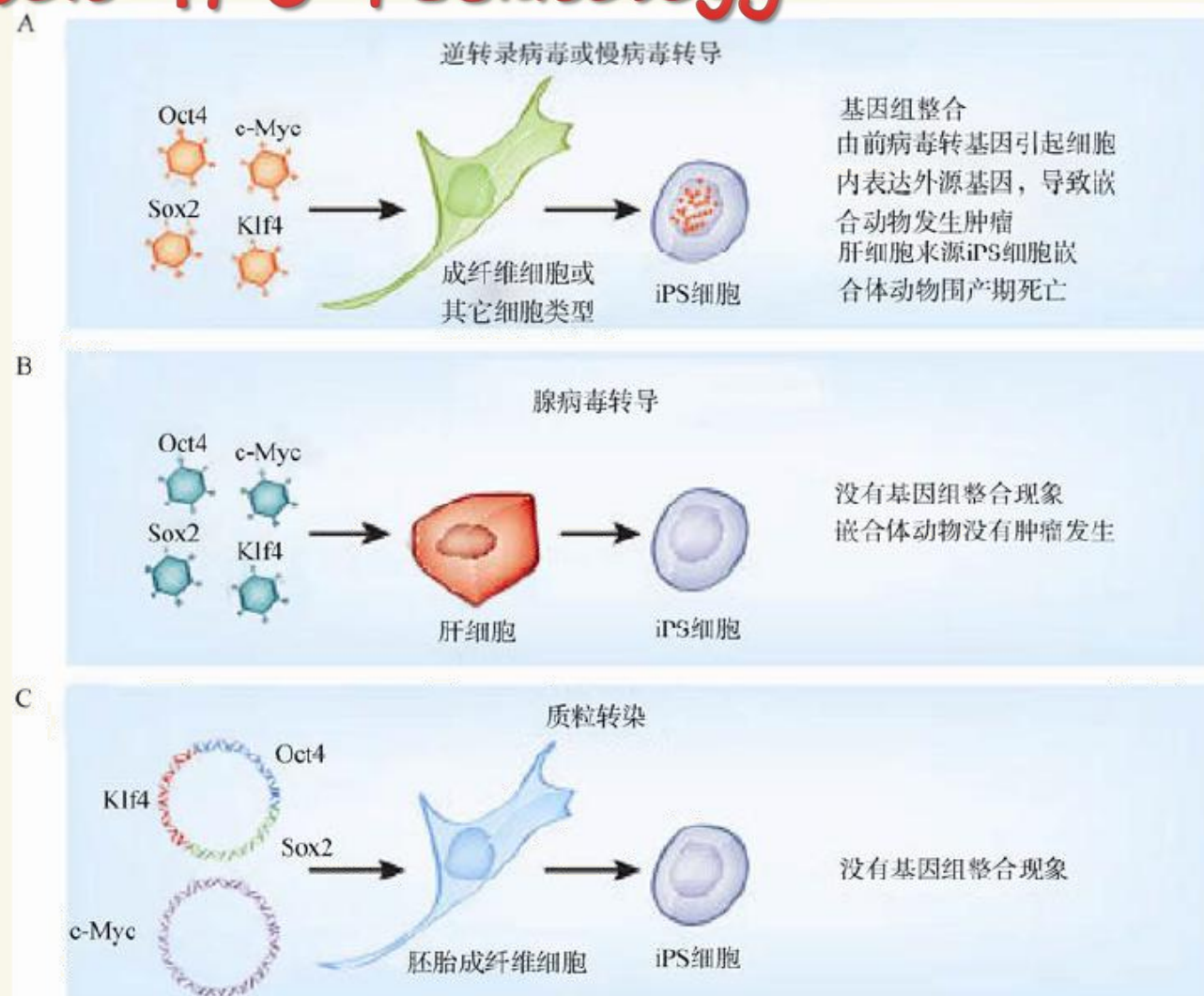
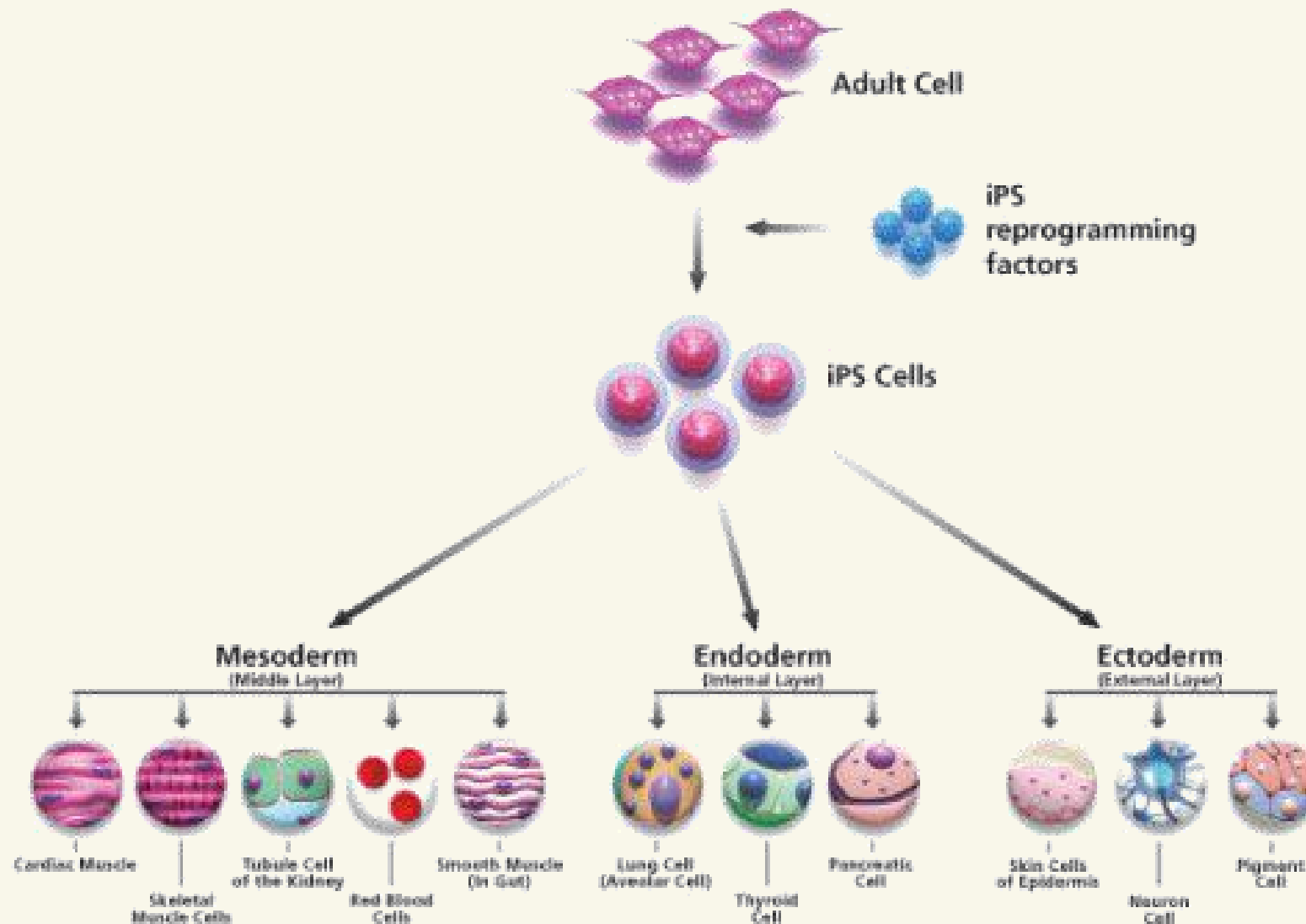


图1 逆转录病毒、腺病毒、质粒转染3种方法诱导iPS细胞<sup>[1,36,37]</sup>

A: 逆转录法; B: 非整合瞬时表达重编程因子法; C: 外源DNA法。





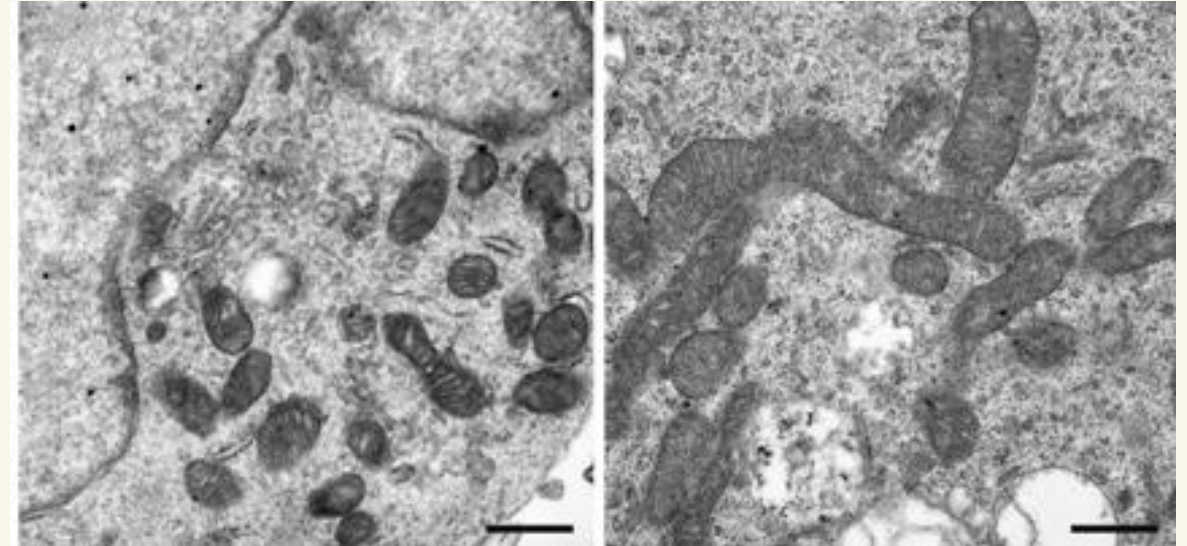
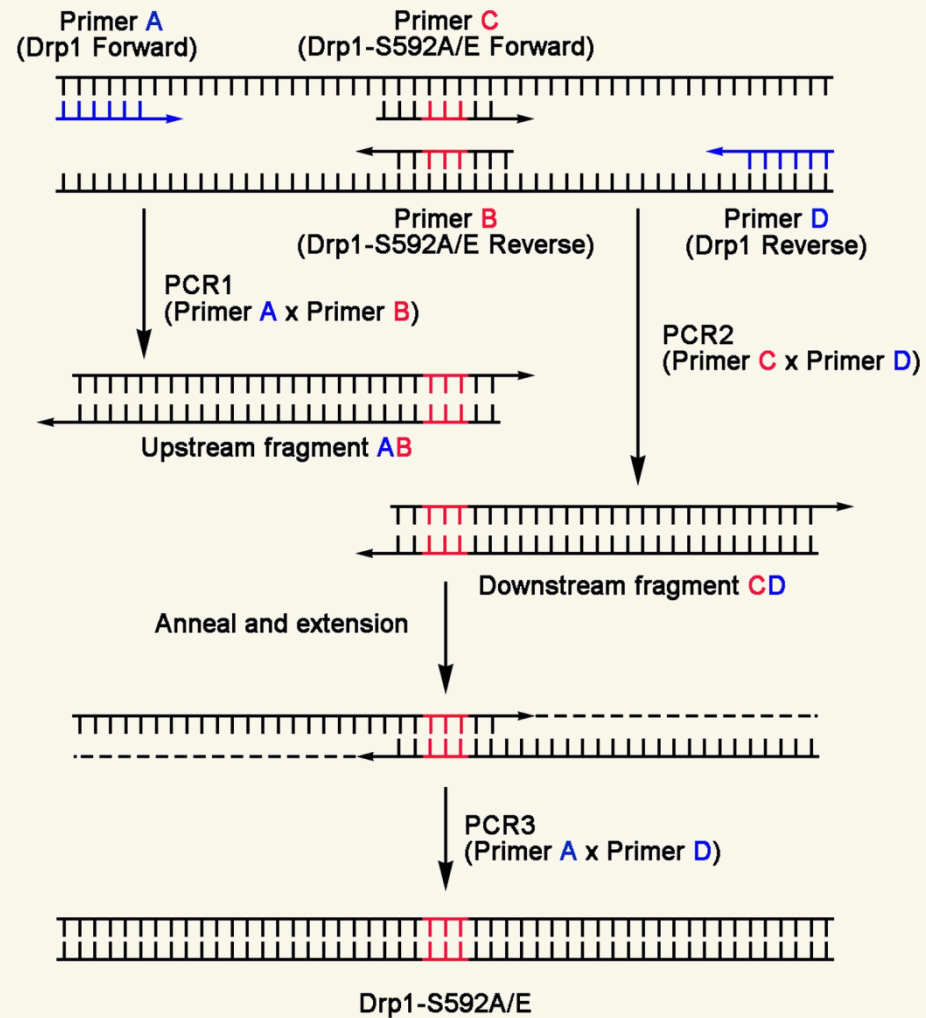
## (2) Mutation

PCR  
Product

Protein phosphorylation:

Proteins could be phosphorylated or dephosphorylated by the action of protein kinases, protein phosphatases, respectively, which plays an important role on cell signal transduction (Ser, Thr and Tyr)

A



B

Drp1	TACGGTCCG <b>S</b> AGGTGTTTTT
Drp1-S592A	TACGGTCGG <b>A</b> GGGTGTTTTT
Drp1-S592E	TACGGTCGG <b>E</b> GGGTGTTTTT

# (1) Genetics & Apparent genetics

## Analysis

### Genetics:

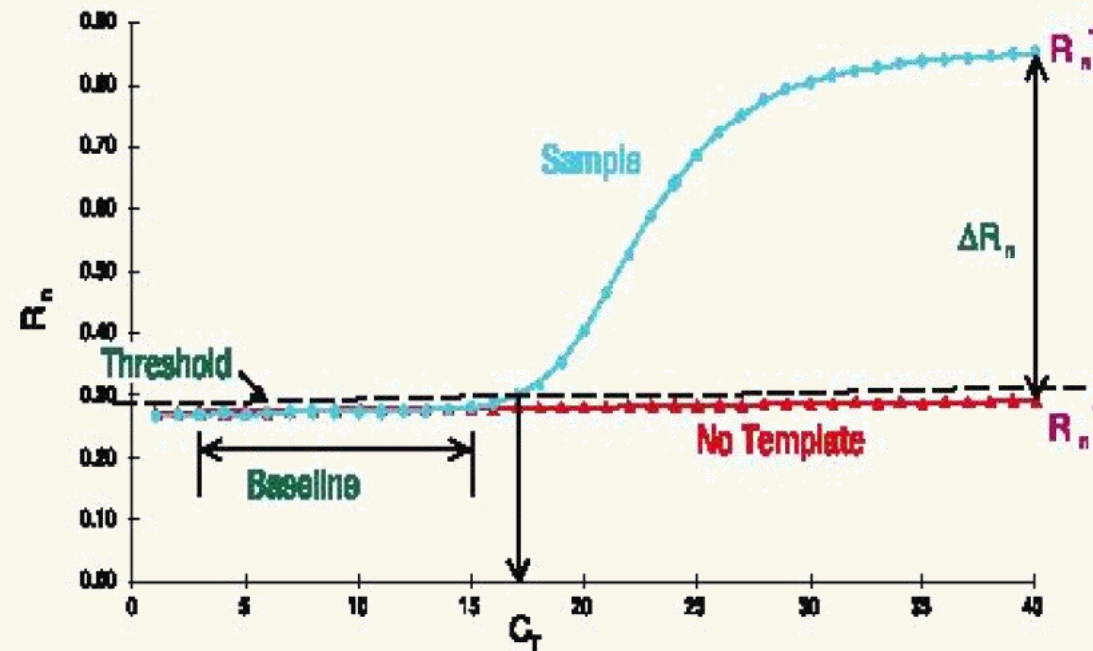
Genetics is the study of genes, genetic variation, and heredity in living organisms. (DNA Sequence)

### Apparent genetics:

DNA methylation, genomic imprinting, maternal effects, gene silencing, RNA editing, etc.

# Analysis

## Absolute Quantification and Relative Quantification (only for real-time PCR)



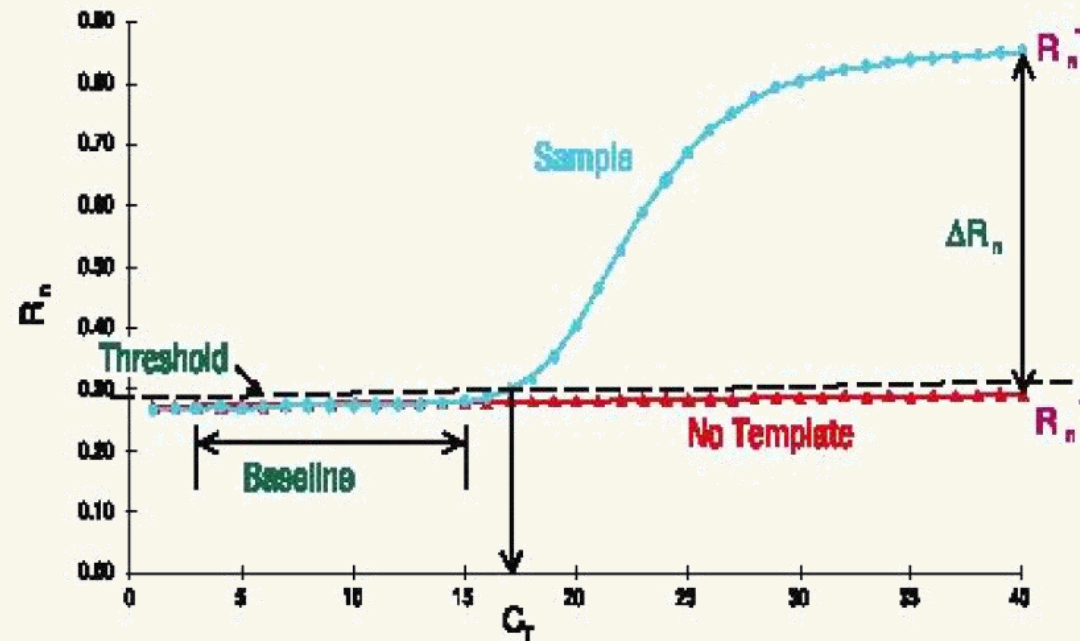
Ct value

图 1.  $C_t$  值的确定

## (二) Absolute Quantification and Relative Quantification

( only for real-time PCR )

The application of PCR: Detect  
the content of PCR products



Ct value

## (二) Absolute Quantification and Relative Quantification

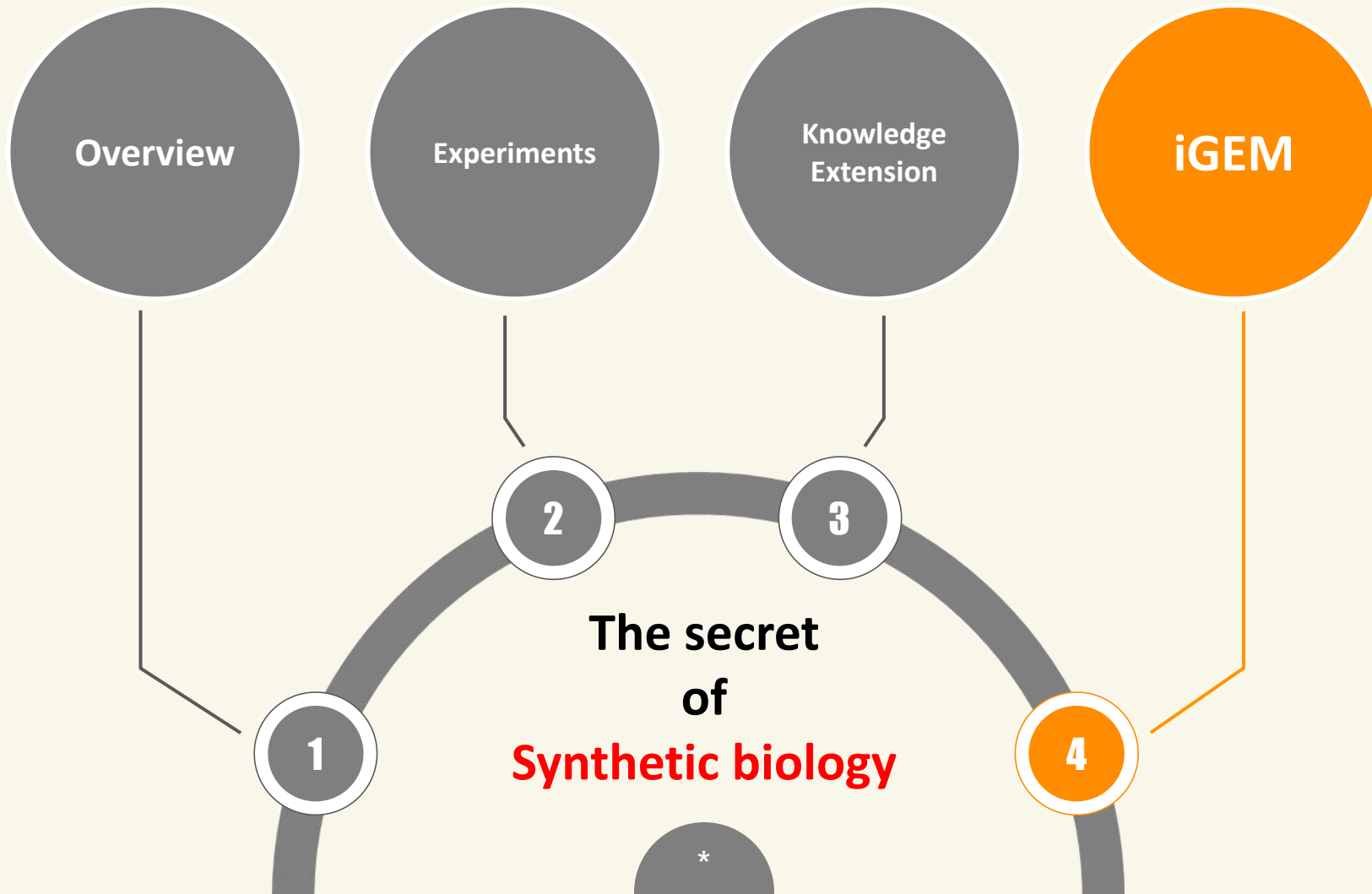
( only for real-time PCR )

**The application of PCR:** Detect  
the content of PCR products

**Absolute Quantification:** Must got a standard curve based on a well-known copy numbers of DNA in the PCR product.

**Relative quantification:** Determine relative proportion of the content of gene of interests in samples.





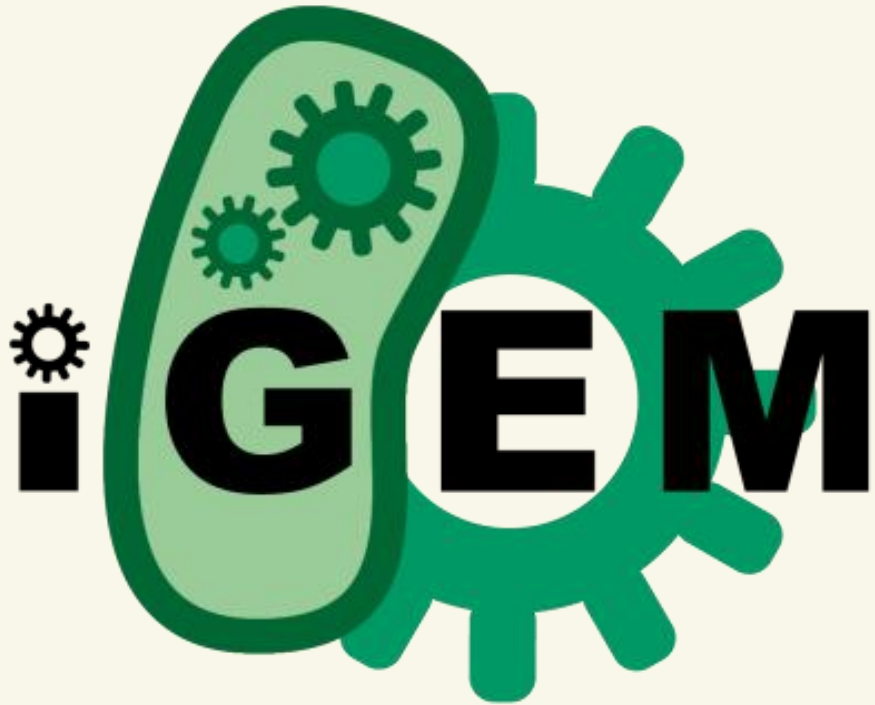


## 4. MSC and iGEM

What's iGEM?



## 4. MSC and iGEM



iGEM, ( International Genetically Engineered Machine Competition ) , is the top competition in synthetic biology.



### 3.Future

**We have provided materials list to Mr Yang,a biology teacher from The 16th Middle School Of Guangzhou, who will suggest school's leader about launching this kind of activity.**

**We are looking forward to receiving approval from school. Moreover, we decide to continue spreading this guide book to other middle schools possessing the instruments and materials needed in the future.**



Thank You  
*The End*



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